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THE EARLY STAGES OF GLOMERULONEPHRITIS*

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Introduction

The structure of the glomerulus in clinical acute diffuse glomerulonephritis has been studied by various investigators and it is generally agreed that the essential lesion is an increase in the number and size of the endothelial cells with resulting capillary obstruction. Polymorphonuclear leukocytes are found in varying numbers among the endothelial cells, but leukocytes alone do not produce complete capillary obstruction except in occasional capillary loops.

Investigators interested in the pathogenesis of human glomerulonephritis have studied clinical examples of the disease in which death occurred shortly after the onset of symptoms. In general they have found appearances similar to, but less prominent than, those occurring in well developed clinical cases.

Gräff, 1916, using Schultze's oxidase reaction, demonstrated a great increase of polymorphonuclear leukocytes in acute glomerulonephritis. He thought that the number of leukocytes in the glomeruli afforded a distinction between simple inflammatory reactions and acute glomerulonephritis.

Gross, 1919, studied the kidneys of a person who died of pulmonary edema a few hours after the onset of symptoms of nephritis. In the glomerular capillaries he noted leukocytes and many large endothelial nuclei embedded in a cytoplasmic network.

Volhard, 1922, 1931, proposed the theory that the primary change in acute glomerulonephritis is a spasm of the afferent glomerular arterioles, the resulting anemia injuring the capillaries and bringing

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about a secondary inflammatory reaction. He based his hypothesis on clinical and physiological considerations and on analogy with eclampsia.

Kuczynski and Dosquet, 1926, supported Volhard's theory with anatomical evidence. In a case which they considered early acute glomerulonephritis they noted edema of the wall of the afferent glomerular arteriole near its entrance into the glomerulus. Leukocytes were also found in the wall of the arteriole. They believed that this structural alteration indicated a primary spastic constriction of the afferent arteriole. The leukocytes in the glomerular capillaries were supposed to have entered through the efferent arteriole. An increase of endothelial nuclei was described.

Volhard's theory has received very little support from anatomical studies. It is, of course, difficult to refute since any spasm of the arteriole present in life would probably relax after death, and one would not expect arteriolar spasm to produce structural changes in the wall of the vessel. In some cases of glomerulonephritis the arterioles show an occasional area of hyaline degeneration in the media, but it is certain that the vast majority show no anatomical changes. They are frequently dilated rather than contracted at the point of entrance into the glomerulus.

Endothelial proliferation does not seem to be related to hypertension. It will be shown later in this paper that severe infections without hypertension show much more endothelial proliferation in the glomeruli than is found in primary hypertension. The high frequency of endothelial proliferation in association with infectious and toxic processes indicates that it is a response to soluble toxic substances rather than to injury from anemia.

Fahr, 1926, restated his belief that glomerulonephritis is caused by soluble toxins that produce a primary endocapillaritis. In a person who died of pneumonia 3 days after the onset of symptoms of nephritis he found a definite endothelial proliferation and many leukocytes in the glomerular capillaries. A careful study of the afferent arterioles showed no lesions of any kind.

Hückel, 1929, studied the kidneys from a case of glomerulonephritis of 30 hours duration. He found the glomerular capillaries filled with polymorphonuclear leukocytes and endothelial cells in a cytoplasmic substance. He agreed with Fahr's conception of primary endocapillaritis.

The prevailing opinion in the literature seems to be in accord with Fahr's conception that the primary change in glomerulonephritis is proliferation of the endothelium of the glomerular capillaries and that the earliest clinical examples of acute glomerulonephritis show this lesion. The simplest interpretation of the endothelial increase is an inflammatory reaction to soluble toxic substances.

We may now raise the question as to whether or not acute glomerulonephritis is a specific disease entity, *i.e.* a disease caused by a specific organism or virus and having characteristic anatomical lesions, such as typhoid fever and tuberculosis. Inasmuch as organisms are not found in the glomeruli the etiological agent can only be inferred from the associated infection in the patient. Nearly all observers are agreed that the associated infectious agent is usually a streptococcus, but both hemolytic and non-hemolytic strains are concerned. There is also strong evidence that pneumococci may occasionally cause glomerulonephritis, and in rare instances other organisms may be concerned. Staphylococci apparently do not produce diffuse endothelial proliferation in the glomeruli to any notable extent although they often produce glomerular abscesses. It may be concluded, therefore, that glomerulonephritis is not caused by a specific etiological agent.

As regards the anatomical lesion, the essential feature is proliferation of the glomerular capillary endothelium. Polymorphonuclear leukocytes are usually present in varying numbers but are not an essential feature. Leukocytes alone rarely, if ever, produce widespread capillary obstruction. Epithelial crescents predominate in certain fulminant cases and may be largely responsible for obstruction of the glomeruli.

In 1926 Clawson, Bell and Hartzell published the observation that a moderate degree of diffuse glomerulitis is frequently found in persons dead of subacute bacterial endocarditis. Since that time I have noted a similar glomerulitis in various acute infectious processes, notably puerperal septicemia. These subclinical forms of diffuse glomerulonephritis were illustrated in my "Text-Book of Pathology" in 1934 (Figs. 217 and 218).

In this paper the study of the finer glomerular structure has been extended to include a large number of infectious and non-infectious diseases. It will appear from this investigation that a diffuse proliferation of the glomerular endothelium occurs in many acute and

chronic infectious processes and in some diseases in which no infectious element was found. There are innumerable transitions between this subclinical glomerulitis and clinical acute glomerulonephritis. The clinical disease shows an increased endothelial proliferation and more pronounced capillary obstruction, but fundamentally it is the same type of reaction that occurs in the subclinical forms.

If this interpretation be correct, *i.e.* that subclinical and clinical acute glomerulonephritis differ only in intensity, it follows that acute glomerulonephritis is not a sharply circumscribed entity and a much broader approach to its etiology and pathogenesis is available.

Material and Methods

In this investigation the kidneys from 865 cases have been examined microscopically (see Table II). A wide range of diseases is included and all age groups are represented. Paraffin sections were stained by the Mallory-Heidenhain technique (azocarmine). This stain was first applied to the glomeruli by McGregor. Since the capillary basement membrane is stained sharply, one may easily distinguish cells within the capillaries (endothelial cells and leukocytes) from the glomerular epithelial cells outside them. The stain works to best advantage on tissues fixed in Helly's or Zenker's fluid, but with slight modifications it is satisfactory after formalin fixation unless the tissues have been in formalin for over a year. Tissues kept in formalin several years may often be stained satisfactorily if the deparaffined sections are treated with Zenker's fluid for 24 hours.

In estimating the degree of endothelial proliferation the number of endothelial and epithelial nuclei in several glomerular loops are counted. When the epithelial nuclei definitely outnumber the endothelial the degree of endothelial proliferation is graded "0," and when the two types of nuclei are of approximately equal number it is graded "+." A definite preponderance of endothelial nuclei is graded "1" and a marked preponderance "2." In Grades 1 and 2 the endothelial nuclei are large and show abundant cytoplasm about them. Grade 3 endothelial proliferation corresponds with the structure seen in typical clinical acute glomerulonephritis. Intracapillary fibers are easily seen in Grade 3, and occasionally in very small amount in Grade 2, but are entirely absent in lower grades of proliferation.

In counting the nuclei within the capillaries polymorphonuclear leukocytes were not included, but mononuclear leukocytes were counted as endothelial cells unless they lay entirely free in the capillary lumen. It is very difficult to distinguish a mononuclear leukocyte from a large endothelial cell when the former is flattened against the capillary wall.

This method of enumerating the endothelial nuclei does not, of course, give the total number of cells in a given volume of glomerular tissue and it assumes that the number of epithelial cells is fairly constant.

It is true as Van Waveren points out that fewer endothelial nuclei are visible in a section when the capillaries are distended than when they are empty. In a normal glomerulus, where the endothelial nuclei are smaller than the epithelial, this may lead to underestimation of the relative number of endothelial cells; but in all forms of glomerulitis the endothelial nuclei are as large as the epithelial, and therefore distention or collapse of the capillaries does not alter the ratio of endothelial to epithelial nuclei.

Grades 1 and 2 of endothelial proliferation are immediately recognizable with the high dry lens. Figures 3, 4, 5 and 6 illustrate what is meant by endothelial proliferation — 0, +, 1, and 2 respectively.

The Structure of the Normal Human Glomerulus

In a normal glomerulus the lobulation is usually indistinct since the interlobular fissures are difficult to see. However, in chronic glomerulonephritis the lobules are often shrunken so that the interlobular fissures become conspicuous (Figs. 1 and 2). In midsagittal sections through the vascular pole of such slightly shrunken glomeruli one sees from three to five fissures that penetrate nearly to the vascular pole. Some of the primary lobules thus formed are subdivided peripherally by fissures which extend from one-third to one-half the distance to the vascular pole and form secondary lobules. Capillary loops bulge from the surfaces of the secondary lobules to form small tertiary lobulations. The tertiary lobules are indistinct in the shrunken glomerulus (Fig. 1) but are easily seen in a normal glomerulus. In tangential sections through a glomerulus one sees small isolated, secondary or tertiary lobules, each composed of one or more capillary loops surrounded by epithelium.

Judged from the appearances seen in sections through different

planes one may conclude that there are from four to six primary lobules of irregular conical shape with their apices near the vascular pole and their wide bases at the surface of the glomerulus. Each primary lobule branches distally into secondary and tertiary lobules.

It is clear that the elaborate separation of the glomerulus into lobules serves the purpose of bringing nearly all the capillaries into contact with the surface. There are few capillaries that are not in contact with the surface epithelium at some part of their circumference, and those in the small peripheral tertiary lobules are usually largely or completely surrounded by epithelium. The glomerular epithelium covers the surface of the glomerulus and lines all the shallow intralobular as well as the deep interlobular clefts. There are obviously no capillary anastomoses across any of the fissures.

The glomeruli in newborn infants are smaller and of less complex structure than those of adults. The primary lobules are smaller and secondary lobulation is much less conspicuous.

The finer structure of the glomerulus is best seen in the small tertiary lobules at the surface. These consist of a few capillary loops closely invested by glomerular epithelium (Fig. 3). The epithelium covers the outer surfaces of the capillaries and fills the interstices between them. The nuclei between capillaries probably all belong to epithelial cells.

The Glomerular Epithelium

The glomerular epithelium is the visceral epithelial layer of the capsular space and is continuous around the vascular pole with the capsular epithelium (the parietal epithelial layer of the capsular space). Both the parietal and the visceral epithelial layers of the capsular space have the same embryonic origin as the cells of the convoluted tubules, since the capsular space is formed by invagination of the glomerular tuft into the expanded end of the primitive tubule. This relation is seen in tubular disease of the kidneys in which one may see hyaline granular degeneration, fatty degeneration and necrosis of the glomerular and capsular epithelial cells when these changes are present in the tubular epithelium.

As pointed out above, the epithelial layer lines all the interlobular and intralobular clefts and penetrates into the lobules between the capillaries. It therefore forms a support for the capillaries as well as an external covering. The epithelial cells are much more conspicu-

ous in some kidneys than in others. In infants the surface layer is columnar or cubical in shape and very conspicuous. In adults the cytoplasm of the epithelial cells is sometimes so abundant that the cells seem to compress the capillaries. More often, however, their nuclei are found in the interstices between the capillaries and sparsely distributed over the surface. Wide areas of the capillary surfaces may appear to be denuded of epithelium, but a careful study will always reveal a layer of epithelial cytoplasm separating the capillary basement membrane from the capsular space (Fig. 3). The surface epithelial layer undergoes postmortem autolysis rapidly, and the number and size of the cells are underestimated in poorly preserved tissue.

The parietal epithelium of the capsular space (the capsular epithelium) plays an important rôle in glomerulonephritis, since it is the source of the epithelial crescents; but the glomerular layer shows chiefly degenerative changes and never proliferates sufficiently to compress the glomerulus.

The Glomerular Endothelium

McGregor, 1929, has described and illustrated the finer histology of the normal glomerulus. In preparations stained by the Mallory-Heidenhain method the endothelial nuclei are easily distinguished from the epithelial by their position on the inner surface of the capillary basement membrane. In a large majority of normal kidneys the structure of the glomerulus corresponds to that shown in Figure 3, which I have called Grade 0. The epithelial nuclei greatly outnumber the endothelial. Practically no cytoplasm is seen about the endothelial nuclei or elsewhere on the inner surface of the basement membrane.

In Table I the degree of endothelial proliferation in 107 normal kidneys is recorded, the cases being arranged by decades. The kidneys classified as normal were from individuals who died of trauma or carbon monoxide poisoning not more than 6 hours after the injury was sustained. The majority lived less than 1 hour after the accident. A further requirement was that there should be no gross or microscopic evidence of any disease other than the fatal trauma or poisoning.

It will be noted from the table that 90 of the 107 cases showed Grade 0 endothelial proliferation, which indicates that the epithelial

nuclei were more numerous than the endothelial. A definite preponderance of epithelial nuclei is illustrated in Figure 3. In 16 of the 107 cases epithelial and endothelial nuclei were present in approximately equal numbers. This type of structure is graded + and is illustrated in Figure 4. Inasmuch as about one-seventh of the normal kidneys showed Grade + structure, this must be accepted as a variation within normal limits.

TABLE I

The Endothelium of the Glomerular Capillaries in Apparently Normal Kidneys *

Decade	No. of cases	Endothelial proliferation		
		0	+	1
1	9	9	0	0
2	8	6	2	0
3	17	13	4	0
4	25	21	4	0
5	17	15	1	1
6	11	9	2	0
7	12	12	0	0
8	6	4	2	0
9	2	1	1	0
Total	107	90	16	1

* In the 0 column the kidneys are listed in which the epithelial nuclei definitely outnumber the endothelial (Fig. 3). Grade + indicates that the epithelial and endothelial nuclei are approximately equal in number (Fig. 4). Grade 1 indicates a definite preponderance of endothelial nuclei (Fig. 5).

In only one instance did an apparently normal kidney show a definite preponderance of endothelial nuclei. This structure is called Grade 1 endothelial proliferation and is illustrated in Figure 5. Although no disease was found at postmortem to account for this endothelial increase, it is probably beyond the limits of the normal variation in structure.

Nussbaum, 1886, demonstrated cell boundaries in the endothelium of the frog's glomeruli. Bensley and Bensley, 1930, were able to see some cell boundaries in human glomeruli by staining with silver; and Zimmermann, 1933, observed cell boundaries in the glomeruli of cats. However, a majority of investigators have failed to demonstrate cell boundaries in the endothelium of the glomerular capillaries although they found them readily in the afferent arteriole.

There is disagreement in the literature as to the number of endo-

thelial nuclei normally present. Langhans, 1885, found only a few endothelial nuclei. Von Möllendorff, 1927, observed in the human glomerulus only a few endothelial nuclei. A little cytoplasm was found about the nuclei but no cell boundaries were demonstrated. Bargmann, 1929, 1931, and McGregor, 1929, agreed with Von Möllendorff that the endothelial cells are few in number, sparsely distributed and greatly outnumbered by the glomerular epithelial cells.

Borst, 1931, found that endothelial nuclei are more numerous than epithelial except in infants and young children where a reverse relation obtains. Borst used a technique which involves boiling of fresh tissue and results in great damage to the cytoplasm of the epithelial cells.

Van Waveren, 1935, used a technique similar to Borst's except for a short preliminary fixation in formalin before boiling. This method gives good pictures of the basement membrane but destroys the cytoplasm of epithelial cells. Van Waveren estimated the number of endothelial and epithelial nuclei in corresponding volumes of glomerular tissue and came to the conclusion that endothelial nuclei always outnumber epithelial. He presents the interesting hypothesis that even in glomerulonephritis the endothelial cells do not increase in number but only in size. It seems, however, that the boiling method damages the epithelial cells so severely that one may be led to underestimate their number. It is also to be noted that the postmortems from which this author apparently obtained his material were performed 36 to 42 hours after death. Ordinarily the surface layer of glomerular epithelial cells has undergone extensive autolysis by this time, and one would not expect to find all of these cells still present.

Van Waveren maintains that the appearances which I have described as glomerulitis do not represent an actual increase of endothelial cells but merely an apparent increase due to contraction of empty capillaries. Empty collapsed capillaries, however, usually show a wavy basement membrane and are easily distinguished from glomerulitis.

Wilbur, 1931, studied the kidneys of 25 apparently healthy subjects dead from accidental causes. He found that endothelial nuclei were from four to six times as numerous as epithelial.

My own observations have been recorded above. The epithelial cells were found to outnumber the endothelial in 90 of 107 normals

and usually the former were much more numerous than the latter. In 16 instances the endothelial and epithelial cells were approximately equal in number. My conclusion is that a definite preponderance of endothelial cells with an increase of their cytoplasm, as shown in Figure 5, represents a glomerulitis. It will be shown presently that the endothelial cells often show a great increase in number and size in infectious and toxic processes, and if one examines the kidneys from a large series of consecutive postmortems he will find that a fairly high percentage of them show more endothelial than epithelial cells in the glomeruli. It appears from Table II that 41.7 per cent of the 865 cases studied showed a definite excess of epithelial over endothelial cells, while in 30.4 per cent the reverse relation obtained. In 27.8 per cent the endothelial and epithelial cells were approximately equal in number. The material studied, however, is not a uniform sample of postmortem material since there is an undue proportion of infectious processes. In a corresponding number of consecutive postmortems the percentage with Grade o endothelium would doubtless be greater.

TABLE II

*Age Distribution of the Endothelial Patterns in All the Cases Studied, Including the Normals**

Decade	Endothelium										Total
	o		+		1		2		3		
	No. cases	Per cent	No. cases	Per cent	No. cases	Per cent	No. cases	Per cent	No. cases	Per cent	
0–10 yrs.	42	79.3	6	11.3	4	7.5	1	2.0	0	0	53
10–20 "	18	30.0	19	31.6	18	30.0	4	6.7	1	1.7	60
20–30 "	34	26.6	34	26.6	46	36.0	12	9.4	2	1.5	128
30–40 "	50	34.0	53	36.0	36	24.5	7	4.8	1	0.7	147
40–50 "	74	42.8	48	27.7	43	24.8	8	4.6	0	0	173
50–60 "	56	47.5	23	19.5	35	29.6	4	3.4	0	0	118
60–70 "	53	46.9	33	29.2	22	19.5	5	4.4	0	0	113
70–80 "	23	41.1	23	41.1	10	17.8	0	0	0	0	56
80–90 "	10	..	2	..	4	..	0	..	0	..	16
90–100 "	1	..	0	..	0	..	0	..	0	..	1
Total	361	41.7	241	27.8	218	25.2	41	4.7	4	0.5	865

* Reading horizontally one sees the number of cases of each endothelial pattern and the percentage of each pattern in the decade. Reading vertically one may compare the frequency of any endothelial pattern in the various decades. Grade o indicates that epithelial outnumber endothelial cells; + indicates that epithelial and endothelial cells are approximately equal in number; 1 indicates a definite preponderance of endothelial cells (Fig. 3); 2 indicates a rather marked glomerulitis but somewhat below the clinical stage; 3 indicates a clinical glomerulonephritis.

The Influence of Age on the Endothelial Pattern

In Table II the distribution of all the cases studied, including the normals, is shown according to the decades and the endothelial pattern. The accuracy of the percentages may be judged by the numbers on which they are based. In the first decade the percentage with Grade 0 endothelium is very high. In infants and young children this endothelial pattern prevails even in association with infectious diseases. The low percentage with Grade 0 in the 2nd, 3rd and 4th decades is no doubt due to the inclusion of a large number of cases of puerperal sepsis and bacterial endocarditis. It is improbable that age influences the endothelial pattern after the first decade.

TABLE III

Distribution of the Endothelial Patterns in the Normals and the Various Diseases that were Studied *

	Number of cases	Endothelium				
		Per cent 0	Per cent +	Per cent 1	Per cent 2	Per cent 3
1. Normals	107	84.1	15.0	0.9	0	0
2. Miscellaneous non-infections	94	55.0	30.8	14.0	0	0
3. Primary hypertension	100	46.0	41.0	11.0	2.0	0
4. Lobar pneumonia	112	51.8	29.5	13.4	5.4	0
5. Acute rheumatic endocarditis	61	44.0	15.0	38.0	3.0	0
6. Acute bacterial endocarditis	37	30.0	27.0	43.0	0	0
7. Subacute bacterial endocarditis	85	3.5	17.6	60.0	16.5	2.4
8. Puerperal sepsis	84	6.0	41.7	38.0	12.0	2.4
9. Pulmonary tuberculosis	73	15.1	45.2	37.0	2.7	0
10. Miscellaneous infections	112	36.6	25.9	29.5	8.0	0

* Explanation as in Table II.

Effect of Disease on Endothelial Pattern

Miscellaneous Non-infectious Diseases (Table III, No. 2): This group includes a large variety of diseases in which infection plays no rôle except as a terminal complication. The diseases included are as follows: old valve defect, 15; malignant tumors (carcinoma of stomach, pancreas, and so on), 19; pernicious anemia, 7; atrophy of liver, 8; diabetic coma, 7; alcoholism, 5; coronary disease, 4; burns, 3; arsenic poisoning, 2; and 1 each of 24 other diseases. The frequency of the various endothelial patterns is shown in Table III, No. 2. A Grade 1 glomerulitis was present in 13 of the 94 cases (14 per cent).

It was present in the following diseases: pernicious anemia, 4 cases (out of 7 examined); old healed valvular heart disease, 5 cases; 1 case each of subacute atrophy of the liver, subacute combined degeneration of the spinal cord, carcinoma of the ampulla of Vater, and right heart failure. Terminal infections may have played a rôle in causing the glomerulitis but this could not be established conclusively.

Primary Hypertension (Table III, No. 3): In this group there were 11 cases of Grade 1, and 2 of Grade 2 glomerulitis. In the 2 cases with Grade 2 glomerulitis death was due to renal insufficiency. The causes of death in the 11 cases with Grade 1 glomerulitis were as follows: myocardial exhaustion with congestive heart failure, 4; renal insufficiency, 2; coronary disease, 2; and 1 case each of cerebral hemorrhage, pyloric obstruction and rupture of the aorta. It is not uncommon to find a definite endothelial increase in hypertension with renal insufficiency — glomerulitis often seems to be an essential part of the renal lesion. Not infrequently patients with primary hypertension die of some complicating infection such as septicemia or pneumonia, and occasionally in such cases clinical acute glomerulitis is found at postmortem; but hypertensives with an obvious terminal infection were not included in this group. The glomerulitis in the 9 cases without renal insufficiency cannot be satisfactorily explained as a result of infection.

We shall now consider the glomerular structure in definite infectious diseases.

Lobar Pneumonia (Table III, No. 4): One-hundred-twelve cases of this disease were studied. It is surprising to find that the frequency of glomerulitis is not significantly greater than in the noninfectious diseases. It is true that there are 6 cases of Grade 2 glomerulitis, but over 50 per cent of the kidneys show the Grade 0 endothelial pattern. No correlation could be found between the degree of endothelial proliferation and the duration of the illness or the age of the patient. Apparently pneumococci do not stimulate the glomerular endothelium to the degree that streptococci do.

It is recognized in the literature that clinical acute glomerulonephritis may follow lobar pneumonia, but postpneumonic nephritis is generally believed to be quite rare. Abrahams, 1920, found that acute nephritis developed in only 2 of 558 cases of typical lobar pneumonia. Eliassow, 1920, described a convincing case of acute

glomerulonephritis in a male 38 years of age, who developed symptoms (albuminuria, edema and moderate hypertension) on the 11th day after the onset of pneumonia. The disease ended in recovery about 6 months later. Seegal, 1935, in a study of 1004 cases of lobar pneumonia found that 7 developed acute glomerulonephritis.

McIntosh and Reimann, 1926, studied renal function during pneumonia. They noted that some previous investigators had found a slight decrease and others a slight increase of kidney function. They studied the elimination of phenolsulphonephthalein after intravenous injection, and also determined the index of urea concentration. The kidneys frequently showed an increased functional ability which began before the crisis and persisted for several days after it. No examples of decreased functional power were mentioned.

Neale, 1928, examined the urine of 287 adult patients with lobar pneumonia. In 3.4 per cent the albumin was +++, in 48 per cent +, and in 47.5 per cent it was absent. No examples of acute glomerulonephritis were found in the 42 postmortem examinations that were made. In 102 cases of pneumococcal infection other than lobar pneumonia, normal urine was found in 46 cases, albumin alone in 45, and albumin casts and blood in 11. In 21 cases of acute pneumococcal infection in children under 7 years of age the urine was normal in 5, contained some albumin in 12, and contained albumin, casts and blood in 4.

Lyttle and Rosenberg, 1929, state that pneumonia in children is frequently followed by nephritis.

Blackman, Brown and Rake, 1931, injected rabbits intravenously with pneumococcal autolysate and intradermally with pneumococci. Eighteen of the rabbits developed generalized edema with ascites. The lesions in the kidneys were interpreted as comparable to human acute and subacute nephritis. These authors also found mild acute and subacute nephritis in 40 to 50 per cent of persons dead of pneumococcal infections. However, there was no anatomical evidence submitted which indicates that any of these renal lesions were true glomerulonephritis. The lesions described were chiefly tubular injuries, thrombosis of glomerular capillaries and occasional epithelial crescents.

Blackman and Rake, 1932, found acute nephritis of considerable intensity in 9.5 per cent of a group of young infants with pneumococcal infections (empyema, organizing pneumonia, otitis media,

meningitis). The diagnoses were made by postmortem examination; no case was recognized as nephritis clinically. They found no cases of nephritis in older children or adults following pneumococcal infections.

Blackman and his associates use the term "nephritis" in a very broad sense to include lesions that are chiefly tubular, and do not restrict it to glomerulonephritis.

The fact that persons suffering with lipoid nephrosis frequently develop pneumococcal peritonitis has led to the belief that pneumococci are responsible for this type of renal disease.

Acute Rheumatic Endocarditis (Table III, No. 5): This group includes two clinical types: (*a*) those dying during the first attack from septicemia or a complicating infection such as pericarditis; and (*b*) those dying from a recurrent acute attack in which valvular insufficiency was a contributory cause of death. Glomerulitis was somewhat more frequent in the first type. It may be seen in Table III that glomerulitis was present in 41 per cent. Although the group is small this percentage seems significantly higher than in the preceding group. In rheumatic endocarditis there are comparatively few bacteria in the circulating blood and one would not expect to find as much glomerular irritation as in bacterial endocarditis.

Acute Bacterial Endocarditis (Table III, No. 6): This group includes primary bacterial endocarditis of less than 6 weeks duration and bacterial endocarditis secondary to some major infection. The duration of the illness is much shorter than in the subacute form. The number of cases is too small to have much significance, but there is a suggestion that infections of this type cause proliferation of the glomerular endothelium.

Subacute Bacterial Endocarditis (Table III, No. 7): In this disease, which is nearly always caused by streptococci, there is usually a prolonged bacteriemia and the glomeruli are exposed to large quantities of bacterial poisons for many months. As might be anticipated, the effects on the glomerular endothelium are very striking. Only 3.5 per cent of the glomeruli show the Grade 0 endothelial pattern, as compared with 84.1 per cent in the normals, and 79 per cent of the cases show glomerulitis. The higher degrees of glomerulitis are numerous, and in 2 instances the typical structure of clinical acute glomerulonephritis was present although it was not recognized

as such clinically. Seven cases were omitted from the table because the glomeruli were so extensively involved with embolic lesions that the endothelial pattern could not be determined. There is no correlation between the number and size of the embolic lesions and the intensity of the endothelial proliferation. Many cases of severe diffuse glomerulitis showed no embolic lesions. The glomerulitis in the cases of acute endocarditis mentioned above is much less intense than in the subacute group, but no definite relation could be established in the subacute group between the duration of the disease and the intensity of the glomerulitis. The glomerulitis may be more pronounced in a case of 2 months duration than in one that lasted over 1 year.

In bacterial endocarditis every transition may be found between kidneys that show the normal Grade 0 endothelial pattern and those that show the structure of typical clinical acute glomerulonephritis. In a large series of these cases one may trace the pathogenesis of the glomerular lesions, and the various stages are illustrated in Figures 4, 5, 6 and 7. The more or less constant presence of streptococci in the blood in this disease over a period of several months would lead us to expect a much higher incidence of clinical acute glomerulonephritis; yet 60 per cent of the cases show only Grade 1 glomerulitis. Evidently the development of the clinical lesion depends on some factor other than the presence of streptococci in the blood stream. Baehr and Lande, 1920, found that 9 of 77 cases of subacute streptococcic endocarditis showed diffuse glomerular damage.

Puerperal Sepsis (*Table III, No. 8*): In this disease, as in subacute bacterial endocarditis, there is a high incidence of glomerulitis, 52.4 per cent. Most of these are Grade 1 glomerulitis; but 12 per cent show the Grade 2 pattern, and there are 2 cases of clinical acute glomerulonephritis. This disease is usually due to streptococci, although other organisms, *e.g.* staphylococci, are occasionally responsible. Peritonitis and bacteremia are the usual fatal complications. As in the case of subacute bacterial endocarditis, there are numerous transitions between mild and severe glomerulitis, and the distinction between subclinical and clinical glomerulonephritis is somewhat arbitrary.

Pulmonary Tuberculosis (*Table III, No. 9*): In this group only those cases are included in which the patient died of chronic pulmonary tuberculosis. In all instances the lungs were extensively

destroyed by cavities and tuberculous tissue. It is probable that the high frequency of glomerulitis, 39.7 per cent, is due to the pyogenic infection in the cavities rather than to any toxic products of the tubercle bacillus. Some of these kidneys contain deposits of amyloid. In a previous publication, 1933, I have called attention to the increase of endothelial nuclei that precedes the deposition of amyloid. It appears from the present study that this endothelial increase is the result of the underlying infection and is independent of the formation of amyloid. Occasionally a clinical acute glomerulonephritis follows pulmonary tuberculosis.

Miscellaneous Infections (Table III, No. 10 and Table IV): This group includes 112 cases of various infectious processes encountered

TABLE IV

*Miscellaneous Infections Arranged According to the Disease and the Degree of Endothelial Proliferation**

Infection	Endothelium				Total cases
	0	+	1	2	
Typhoid fever	3	2	0	0	5
Septicemia	5	4	4	1	14
Peritonitis, appendicitis	8	6	7	2	23
Pyelonephritis	0	0	2	0	2
Acute and chronic suppuration	3	12	9	1	25
Diphtheria	6	1	0	1	8
Scarlet fever	6	0	0	0	6
Bronchopneumonia	2	1	1	1	5
Endarteritis	0	0	1	0	1
Septic sore throat	1	1	2	0	4
Pericarditis or pleuritis	1	2	2	0	5
Meningitis	6	0	3	0	9
Acute hepatitis	0	0	0	1	1
Lupus erythematosus	0	0	1	1	2
Purpura hemorrhagica	0	0	0	1	1
Enterocolitis	0	0	1	0	1
Total	41	29	33	9	112

* Explanation as in Table II.

in a series of consecutive postmortems. No instance of clinical acute glomerulonephritis following an infection was encountered in this series of postmortems, but clinical glomerulonephritis is commonly related to such infections. In a group of 57 cases of clinical acute glomerulonephritis collected from a series of 23,000 postmortems there were 25 cases that followed miscellaneous infections of the type

listed in Table IV: 37.5 per cent of this group show glomerulitis, Grades 1 and 2.

In Table IV the group is arranged according to the disease and the endothelial pattern. The surprisingly high incidence of the Grade 0 endothelial pattern is due to the inclusion of 27 cases in children under 10 years of age, 22 of which showed the normal Grade 0 structure. If these 27 cases are excluded the percentage of Grade 0 type drops from 36.6 to 22 per cent, and is then more comparable to the other groups shown in Table III which are chiefly adults. It is to be noted that diphtheria and scarlet fever do not have much effect on the endothelium. Fahr, 1916, found only 1 case of acute glomerulonephritis from postmortem examination of 110 cases of diphtheria. It is known that glomerulonephritis is rarely found in persons who die during an attack of scarlet fever.

The Relation of Endothelial Proliferation to Albuminuria

In the 246 cases included in Table V the urine was examined at some time during the fatal illness and usually only a few days before

TABLE V

The Relation of Endothelial Proliferation to Albuminuria

Endothelium	Albuminuria				Total
	0 or trace		+ to ++++		
	No. of cases	Per cent	No. of cases	Per cent	
0	47	74.6	16	25.4	63
+	41	67.2	20	32.8	61
1	74	71.8	29	28.2	103
2	10	52.6	9	47.4	19
Total	172		74		246

death. The cases in which the urine contained no albumin or only a trace are listed together. If this group be subdivided, there are 100 with no albumin and 72 with a trace. The cases in which albuminuria was graded + to ++++ are listed together, since these are only rough estimations of the amount of albumin. It is obvious that there is no relation between the presence or the amount of albumin and the degree of endothelial proliferation. A few cases with Grade 0 endothelium showed heavy albuminuria, and some cases with Grade 2 proliferation showed no albumin. It may be argued that this

is evidence that the endothelial proliferation is not an inflammatory reaction, but it is well established that albuminuria depends on some injury of the capillary endothelium which makes it permeable to protein. There is more albumin in the urine in lipoid nephrosis, which often shows little or no endothelial proliferation, than in glomerulonephritis with a pronounced endothelial increase.

Cloudy swelling of the kidneys likewise shows no direct relation to increase of endothelial cells, since it may be as pronounced in those with the Grade 0 endothelial pattern as in those with Grade 2. However, those with the Grade 2 pattern always showed cloudy swelling.

The Rôle of the Polymorphonuclear Leukocyte

Gräff, 1916, emphasized the importance of polymorphonuclear leukocytes in acute glomerulonephritis and expressed the opinion that the number of these cells in the glomerular capillaries affords a distinction between simple inflammatory irritation and true nephritis. In clinical acute glomerulonephritis the polymorphonuclears are often conspicuous and are partly responsible for capillary obstruction when they are distributed among the endothelial cells; but when present alone they rarely cause permanent capillary obstruction since they do not become attached to the capillary wall.

In the subclinical forms of glomerulonephritis which are discussed in this paper, the polymorphonuclears are sometimes present in considerable numbers but usually they are inconspicuous. In estimating the degree of endothelial proliferation these leukocytes were, of course, not enumerated. The leukocytes probably accumulate in the capillaries because the capillary walls have been injured and they may also be held mechanically in capillaries that are partly obstructed by endothelium.

Focal Glomerulonephritis

The embolic glomerulonephritis associated with bacterial endocarditis is a well recognized type. Clinically it is characterized chiefly by hematuria; anatomically there are focal lesions usually considered embolic in origin. But in all probability these focal lesions are thrombotic and proliferative in character and not embolic. They are apparently due to the lodgement of bacteria in the capillary tufts but they are not infarcts (Bell, 1932). They occur frequently in the absence of endocarditis.

Aside from this so-called embolic type associated with endocarditis, focal glomerulonephritis is ill-defined both clinically and anatomically. Clinicians often diagnose as focal glomerulonephritis the transitory hematuria that sometimes accompanies tonsillitis and other infections when no hypertension, edema or renal insufficiency develops. Thus Werboff, 1928, speaks of hematuria accompanying tonsillitis and appendicitis as due to focal glomerulonephritis. Baehr's benign hemorrhagic nephritis, 1926, evidently belongs in this category. The underlying pathology of these transitory hematurias is not known with certainty, but it is probably glomerular bleeding from ruptured capillaries.

There is no clinical condition other than transitory bleeding from the parenchyma of the kidney that can be interpreted as focal glomerulonephritis. Postmortem studies show that albuminuria developing during the course of an infection is due to diffuse and not to focal glomerular injury.

Fahr uses "focal glomerulonephritis" in a pathological sense to include thrombosis or necrosis of individual capillary loops. Only a few glomeruli may be involved and only a part of the affected glomerulus is obstructed. Apparentl} no constant clinical picture is associated with such focal lesions. Fahr believes that focal glomerulonephritis is due to the lodgement of bacteria in the glomerulus, while diffuse lesions are caused by soluble toxins.

In this series of 865 cases capillary thromboses were rarely seen except in association with endocarditis. Occasionally a few glomeruli show Grade 1 or 2 glomerulitis of a diffuse type when all the others are normal, and usually there are some normal glomeruli when the great majority show glomerulitis. Even in clinical acute glomerulonephritis one may find a few normal glomeruli. A glomerulitis may be focal in the sense that only a small proportion of the glomeruli are involved.

The Significance of Endothelial Proliferation

It is concluded from the foregoing studies that endothelial patterns o and + are normal and that the o type occurs much oftener in the first than in subsequent decades. A large variety of infectious and toxic processes irritate the glomerular capillaries and cause an increase in the number and size of the endothelial cells. The most pronounced endothelial reactions result from severe streptococcic in-

fections, notably subacute bacterial endocarditis and puerperal sepsis. In these infections the endothelial reaction often approaches and sometimes reaches the intensity that is found in clinical acute glomerulonephritis. It appears that a wide variety of irritants produce glomerulitis of a subclinical type and it is only when a definite capillary obstruction is produced that the clinical symptoms of acute glomerulonephritis develop.

Subclinical glomerulitis differs from clinical acute glomerulonephritis only in the extent of the endothelial proliferation. The fundamental pathological reaction is endothelial proliferation in both conditions; and it seems justified, therefore, to consider subclinical glomerulitis as an early stage of clinical glomerulonephritis. The numerous transitions between the two diseases and the similar etiology also support this interpretation. Cases of acute glomerulonephritis that terminate in complete healing may possibly resemble the severe subclinical forms more than they resemble the fatal acute cases.

The Nature of the Endothelial Reaction: The usual interpretation of the endothelial reaction is that it is a proliferative inflammation, *i.e.* the increase in the number of cells is due to division of preexistent endothelial cells. It is recognized that the endothelial nuclei become larger and that the amount of cytoplasm about them increases greatly. The objection to this interpretation is that no mitoses are to be seen in the endothelial cells. Numerous investigators have confirmed the absence of mitoses. In this study of subclinical glomerulitis no mitoses were seen. One is therefore forced to conclude that if cell division actually occurs it is largely of the amitotic type.

Another theory that merits consideration is that the increase of cells is due to the lodgement of mononuclear leukocytes in the capillaries. It is difficult to distinguish mononuclear leukocytes from endothelial cells unless the former lie free in the lumen of the capillary. In Figures 5 and 6 there are some cells that are obviously mononuclear leukocytes and there are others that may belong to this group. In fact the study of glomerulitis of Grades 1 and 2 brings out considerable evidence that at least some of the increase of intracapillary cells is due to the lodgement of mononuclear leukocytes.

A third theory suggested by Van Waveren is that the endothelial

cells increase only in size and not in number. He explains the appearances of glomerulitis, which I have described, as due to contraction of the capillaries. He is also inclined to believe that true glomerulonephritis may be explained similarly as merely an increase in size of endothelial cells. The drawings (Figs. 3–8) were all made at the same magnification and most of the capillaries are distended, except in Figure 4. It seems incredible that these appearances could all be due merely to increase in the size of the endothelial cells.

We may conclude that glomerulitis is due in part to enlargement of the preexistent endothelial cells and in part to lodgement of mononuclear leukocytes, but endothelial proliferation is probably the most important feature of the reaction.

To what extent is glomerulitis a reversible process? No definite information is available on this problem. On theoretical grounds we may believe it reversible until an intensity is attained that results in the formation of hyaline intracapillary fibers between the cells. The formation of fibers leads to fixation of the cells and permanent obliteration of the capillary. The presence of intracapillary fibers may be used to distinguish the clinical from the subclinical stage of glomerulitis.

If one accepts the theory I have sought to establish in this paper that subclinical glomerulitis differs from clinical glomerulonephritis only in intensity, a broader approach to the etiology of glomerulonephritis is available. A large group of infectious and toxic processes is concerned in the etiology of the disease. The glomerular capillaries are injured probably by various toxic substances. Sensitization to bacterial or other protein may play an important rôle, but it is unnecessary to assume that sensitization is essential in the development of the lesion. Masugi, 1933, has shown that glomerulonephritis develops readily in a sensitized animal when the antigen is injected into the renal artery, but this experiment is about the same as the Arthus phenomenon, and is not duplicated in the clinical development of nephritis. The cases of acute glomerulonephritis that develop within a week after the onset of an acute infection are not easily explained as a result of hypersensitiveness. A widespread sensitization to bacterial protein must be assumed if one is to explain subclinical glomerulitis on this basis.

Summary and Conclusions

A microscopic study of the kidneys was made in 107 cases of death from accidental causes, in 194 cases of death from non-infectious diseases, and in 564 cases of death from various infectious processes.

In the 107 normals the glomerular epithelial cells definitely outnumbered the endothelial in 84.1 per cent, the endothelial outnumbered the epithelial cells in only 1 instance (0.9 per cent), and the two types of cells were approximately equal in number in 15 per cent. It was concluded that a definite preponderance of endothelial over epithelial cells represents a glomerulitis.

A Grade 1 glomerulitis was found in 14 per cent of non-infectious processes.

In lobar pneumonia glomerulitis was found in only 18.8 per cent, but in the other infectious groups it varied from 37.5 to 78.9 per cent.

The highest incidence of glomerulitis was found in puerperal sepsis (52.4 per cent) and subacute bacterial endocarditis (78.9 per cent).

It is evident that a variety of toxic substances, especially those derived from streptococci, may irritate the glomerular capillaries and produce an increase of endothelial cells.

In a Grade 2 glomerulitis the glomerular capillaries are filled with cells and occasionally a few intracapillary fibers are present. The distinction from clinical glomerulonephritis is somewhat arbitrary.

The glomerulitis is probably due chiefly to endothelial proliferation, but the lodgement of mononuclear leukocytes in the capillaries seems to play a rôle of some importance.

There is no relation between the presence or the amount of albumin in the urine and the degree of endothelial proliferation.

There is no anatomical basis for a diagnosis of focal glomerulonephritis except in instances of transitory glomerular bleeding not associated with symptoms of nephritis, and in cases of bacterial endocarditis.

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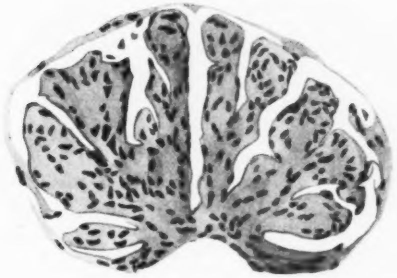
DESCRIPTION OF PLATES

Plate 129

Fig. 1. Glomerulus from chronic glomerulonephritis. This is a stage just preliminary to hyaline degeneration. The shrinkage of the lobules accentuates the interlobular septa. Drawing. Low magnification.

Fig. 2. Glomerulus from chronic glomerulonephritis. The shrinkage is not so great as in Fig. 1, and secondary lobules are more distinct. Drawing. Low magnification.



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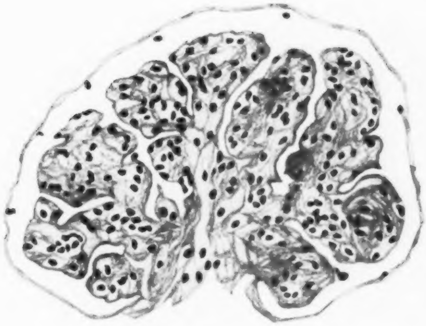

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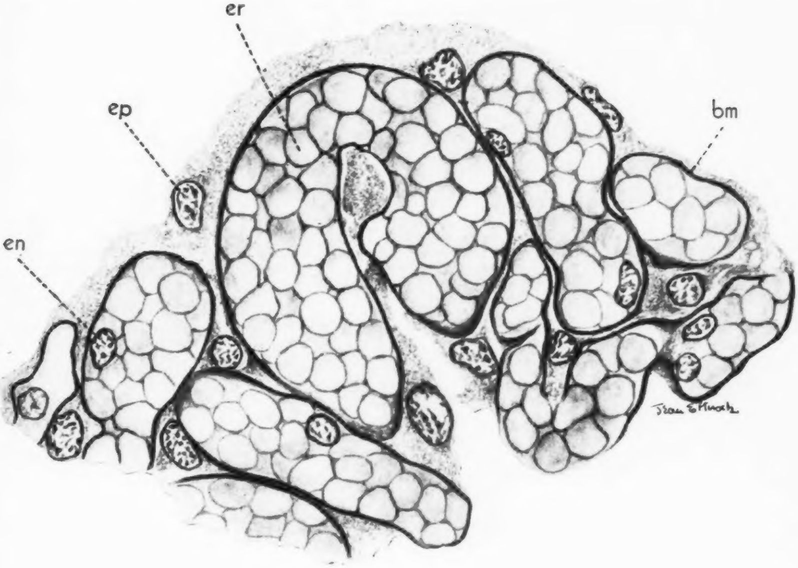
PLATE 130

FIG. 3. Lobule of a glomerulus showing the normal, Grade 0, endothelial pattern. The capillaries are distended. Note that epithelial outnumber the endothelial nuclei. From a case of influenzal pneumonia.

bm = basement membrane of capillary; en = endothelial nucleus; ep = epithelial nucleus; er = erythrocyte. Mallory-Heidenhain stain. Drawing × 1200.

FIG. 4. Lobule of a glomerulus showing the normal, Grade +, endothelial pattern. Note that the endothelial and epithelial nuclei are approximately equal in number. The capillaries are empty of erythrocytes but are not collapsed. The basement membrane is somewhat wavy because of the absence of distention. From a case of puerperal septicemia.

Lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing × 1200.



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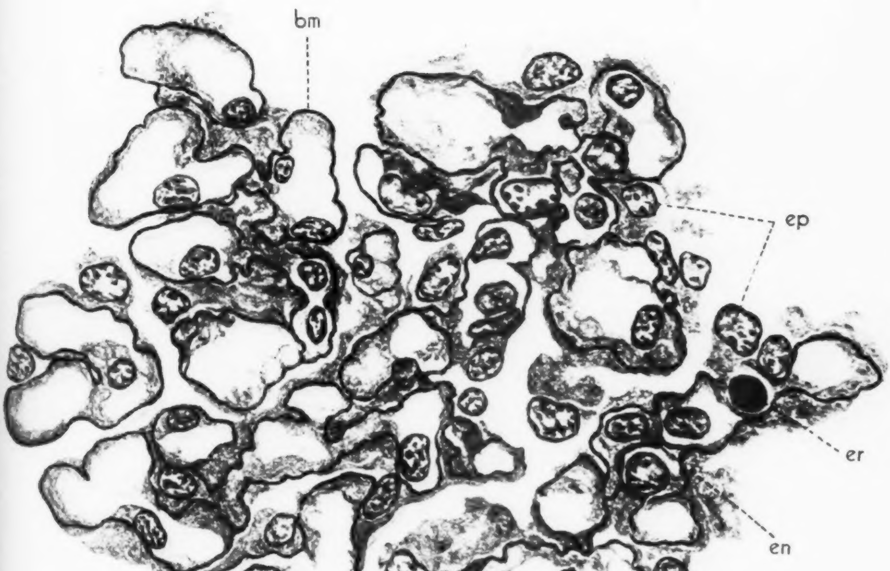

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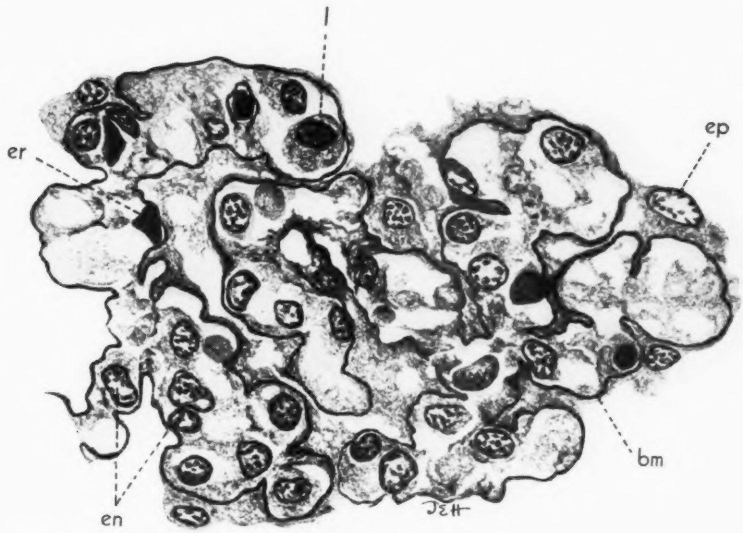
PLATE 131

FIG. 5. Lobule from a glomerulus showing Grade 1 glomerulitis. The endothelial cells definitely outnumber the epithelial and have produced a partial capillary obstruction. Nearly all the cells within the capillaries appear to be of endothelial origin, but one definite mononuclear leukocyte is shown. From a case of septicemia.

l = mononuclear leukocyte. Other lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing × 1200.

FIG. 6. Lobule from a glomerulus showing Grade 2 glomerulitis. The capillaries are somewhat more closely packed with cells than in Fig. 5. A few erythrocytes are seen which are distorted by pressure. An occasional definite mononuclear leukocyte is seen, and the cells with indented nuclei may be leukocytes. From a case of septicemia.

l = mononuclear leukocyte. Other lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing × 1200.



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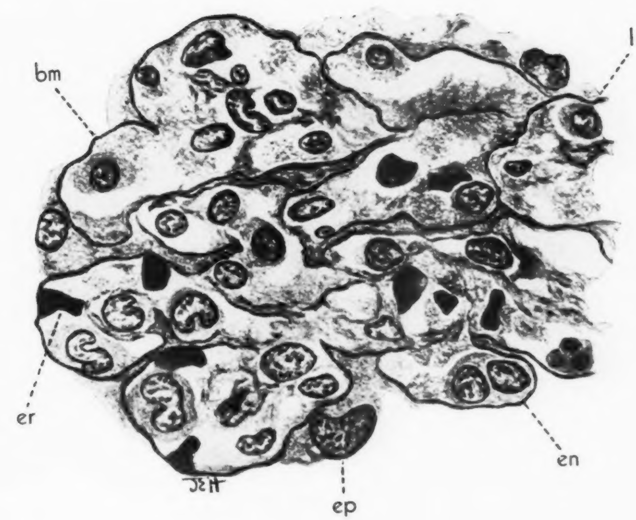

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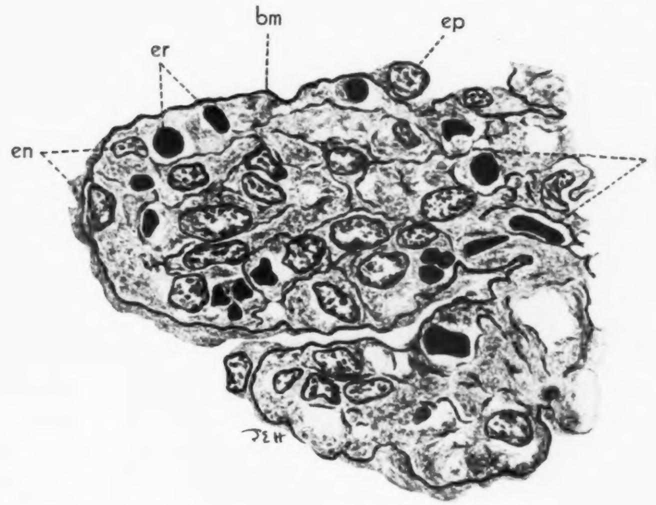
PLATE 132

FIG. 7. Lobule from a glomerulus showing the Grade 3 endothelial pattern. This is clinical acute glomerulonephritis in an early stage. Death from an associated infection. The capillaries are greatly distended.

Note the intracapillary fibers, f. Other lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing × 1200.

FIG. 8. Lobules of glomerulus from a typical case of acute glomerulonephritis, more advanced than in Fig. 7. Death from uremia.

Note numerous intracapillary fibers, f. Other lettering as in Fig. 3. Mallory-Heidenhain stain × 1200.



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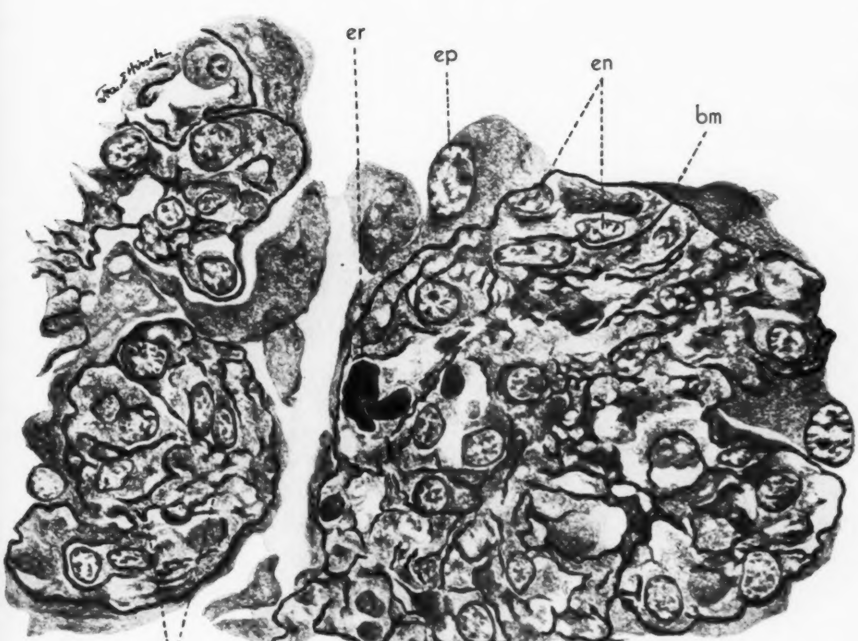

8

THE INTERPLAY OF THE CELLS OF THE HEMATOPOIETIC TISSUES IN RABBITS INFECTED EXPERIMENTALLY WITH THE TUBERCLE BACILLUS*

THE ORIGIN OF THE MONOCYTE CONSIDERED *

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The hematopoietic tissues are in essence mobile, with quasi-fixed portions which serve to manufacture the various cell types that eventually enter into and determine the composition of the circulating blood (the mobilized portion of the system). In postembryonic life specialization of blood cell production occurs in various locations in the body, and the products of widely separated regions of hematopoiesis are brought together through the lymph- and blood-vascular systems. The specialization of blood cell production has led to the designation of the following "systems" of hematopoiesis: (1) myeloid system (bone marrow); (2) lymphoid system (lymph nodes, spleen and scattered lymphoid aggregations, as in the gut); and (3) reticulo-endothelial system (the system of the cell of many aliases, some of which are histiocyte, monocyte, macrophage, endothelial leukocyte, resting wandering cell, and so on). Aschoff[1] limits this system to the spleen, liver, lymphoid tissue and bone marrow, while by others it is extended to include the whole lymph- and blood-vascular system. While the "pigeon-holing" of the blood-forming apparatus into "system" of "fixed" tissue serves a useful purpose, the fact that the hematopoietic tissue is essentially mobile should not be forgotten.

It is apparent that nature has evolved the composition of the blood so that under normal physiological conditions a functional interplay of the different cell types can occur. With the advent of pathological processes extensive alterations may occur in the cellular composition of the blood. The variations from normal depend in large part on the type, extent and gravity of the damage produced by a viable (bacterium, malaria plasmodium, trichina, and so on) or by a chemical (croton oil, and so on) agent. Seldom, if ever, is the alteration confined to one cell type in the blood. And behind the

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variations in the circulating blood changes occur in the factories that produce the blood cells. It becomes obvious then that for a sufficiently broad and inclusive comprehension of the functional interplay in the hematological response to any pathological process representative samples from all of the "systems" of hematopoiesis should be carefully studied. Such examinations should be made serially during the evolution of the pathological process under consideration to determine the possible significance of single observations to the picture as a whole.

It is the purpose of this report to present the conditions found in the hematopoietic tissues during the evolution of the type of tuberculous infection that invariably caused death and to discuss the cellular interplay observed. For ease of presentation the changes found will be given under the headings of "systems."

Methods and Material

We have used the rabbit as the animal for our experiments. During the study, which has extended over a 5 year period, over 100 animals have been used. In those experiments where the different stages of tuberculosis were to be studied, groups of 12 animals were inoculated intravenously with the same dosage of either virulent avian or bovine tubercle bacillus on the same date. The rabbits were then sacrificed in pairs at 24 hours, 5, 10, 14 and 21 days after inoculation, one pair of animals always being left to succumb to the infection. Twelve rabbits were inoculated intravenously with a recently isolated and highly pathogenic strain of *Staphylococcus aureus*. Of these, 2 were sacrificed at the end of 24 hours and the remainder were allowed to die of the infection. All but 2 of the animals died within a week. One lived 16, and another 20 days.

For the data on the changes in the hematopoietic tissues in vaccinated rabbits we used material from other experiments in which over 50 rabbits were used. These animals were vaccinated in groups of 6 to 12 by inoculating human tubercle bacilli (H37) subcutaneously, or by the intravenous injection either of living BCG or of heat-killed virulent bovine bacilli. Two to 6 months were allowed to elapse following the last dosage of vaccine before living virulent bovine bacilli were injected intravenously. Following the injection of the living virulent bacilli the same procedure was followed as for the non-vaccinated rabbits.

The dosage of tubercle bacilli ranged from 0.01 to 5 mg. The photomicrographs shown in the plates were from experiments in which the larger dosage of bacilli was used. The same general changes occurred when smaller amounts of bacilli were used, but the intense acute response of the hematopoietic tissue to the larger dosage rendered tissues from such animals superior for purposes of illustration.

Frequent leukocytic counts were done on all of the animals, both prior to and after inoculation, as can be seen from the tables in the text. The animals chosen to show the leukocytic picture were representative of the groups from which they were taken and had all succumbed to the infection.

Complete autopsies were done on all of the animals used in the experiments and histological preparations were made on all organs. As representative of the hematopoietic tissue, samples from the following were routinely examined: (1) myelogenous system (femur bone marrow); (2) lymphoid system (spleen, mesenteric lymph nodes, and vermiform appendix); and (3) reticulo-endothelial system (the liver, since it is supposed to be the most important portion of this system, aside from the "reticular cells" of the myelogenous and lymphoid tissue).

Results Obtained

Myelogenous System (Femur Bone Marrow)

The cellularity of the femur marrow varies to a considerable extent in different rabbits under normal conditions so that a fixed normal cannot be established. In general, the central portion of the marrow is composed largely of fat cells with the portion adjacent to the bone being the active blood-forming part. The width of this peripheral rim shows considerable individual variation. Occasional, small isolated islands of hematopoietic tissue (Fig. 1) are present in the central area. Mitoses are occasionally found in the peripheral area but have not been observed in the central portion.

At the end of 5 days after tubercle bacilli had been inoculated an increase of hematopoiesis (Fig. 2) was demonstrable in the central portion of the bone marrow. Here the main increase was in immature undifferentiated cells, in maturing myelocytes and in megakaryocytes. Occasional mitotic figures were present in the undifferentiated cells. Mitoses were more frequent than normal in the

peripheral zone where the general cellular picture was similar to that in the central portion of the marrow. Mature segmented neutrophiles were scarce.

At 10 days (Fig. 3) a considerable hyperplasia in the central portion of the marrow was evident. The marrow tissue showed widely separated fat cells throughout. The majority of the cellular increase was composed of immature undifferentiated cells. Mitotic figures were easily found in these cells throughout the marrow. Mature cells of both the myelocytic and the megakaryocytic series were proportionally reduced. No definite tubercles were present, although tubercle bacilli in individual monocytes could be demonstrated. The capillary endothelium appeared normal.

At 14 days (Fig. 4) a still greater hyperplasia had occurred. The fat cells were very widely separated, as can be seen in the photograph where these cells appear as clear spherical spaces. Mitoses were still abundant and undifferentiated cells predominated. Megakaryocytes of the large mammalian type, some of which were emigrating (Fig. 5), were definitely increased in number. Maturing myelocytes were more abundant than at 10 days but segmented neutrophiles were scarce. There was no line of demarcation between the central and peripheral portions of the marrow. Isolated, well defined monocytic tubercles were in evidence. They were more numerous in the peripheral portion of the marrow, as shown in the illustration. These tubercles did not appear to be connected definitely with the blood vessels. They were composed almost wholly of monocytes which were in a good state of preservation. Mitotic figures in the tubercles were found (Fig. 6, in the right upper quadrant) but they were rare. The hematopoietic tissue surrounding the tubercles was in an active state of proliferation, mitoses being frequently encountered.

At 3 weeks (Fig. 7) in avian tuberculous infection a most extensive tuberculosis of the marrow was present. Such extensive involvement was not observed with the bovine type of organism. The tuberculous area of involvement had the appearance of a large continuous sheet of monocytes, the picture of small isolated tubercles, as seen in Figure 4, having largely disappeared. Tubercle bacilli were numerous. Mitotic figures in this area were rare. Giant cells (tuberculous) were rare. Hematopoiesis was active outside of the tuberculous areas. In the non-tuberculous areas the cellular picture was essen-

tially the same as at 14 days. Where the tuberculous tissue and the hyperplastic marrow tissue joined (Fig. 8) the monocytes were in a good state of preservation. Deeper in the tuberculous area (Fig. 9), toward the center of the marrow, there were areas where considerable necrosis of monocytes had occurred. In many of these latter areas a slight to moderate invasion of neutrophiles (the irregular deeper staining nuclear structures seen in Fig. 9) had taken place.

This completes the essential changes usually found in the marrow in the acute tuberculous infection. The large majority of rabbits died of the infection, whether of avian or bovine type, within 4 to 5 weeks. The marrow picture did not differ essentially in these animals from the condition seen at the end of 3 weeks. An occasional rabbit inoculated with the bovine tubercle bacillus lived for 2 months. Rarely such an animal might show tuberculous foci with caseous centers (Fig. 12). This condition was more often found when rabbits were given a smaller dosage of bovine bacilli and lived for several months, although even here such findings were infrequent. It was of interest, however, that this type of lesion, which was so frequently seen in the lung and kidney, could also occur within the bone marrow.

In *vaccinated* rabbits inoculated with 1 mg. of virulent avian or bovine tubercle bacilli intravenously, the marrow changes were the same within the 3 week period, as observed in non-vaccinated rabbits, except that the hyperplasia appeared to be more rapid. Some of the vaccinated reinfected rabbits lived for several months. The condition of the marrow at the time of death of these reinfected, chronically ill animals was of interest. It was uniformly devoid of fat and congested (Fig. 10). It was much less cellular than at the height of hyperplasia (2 to 3 weeks), but the hematopoietic tissue was still notably active in the production of cells, as shown from the frequency of mitotic figures. In Figure 11 six mitotic figures could be determined with certainty under the microscope. In these marrows tuberculous lesions were rare. When present (Fig. 10), they showed circumscribed areas of monocytes, occasional Langhans' giant cells, and moderate to intense lymphocytic infiltration. These marrows showed considerable variation in their megakaryocytic content. In some, megakaryocytes were abundant; in others, they were not increased over normal. The outstanding feature of these mar-

rows was the preponderance, in all specimens examined, of myelocytes and maturing neutrophiles.

In the study of all the marrow tissues an impression was gained that the erythrogenic tissue was at times definitely increased. This, however, had to remain only as an impression since the outstanding marrow hyperplasia was on the undifferentiated cell, myelocytic and megakaryocytic side.

While it is the purpose of this paper to present the changes observed in tuberculous infection, it is not amiss to cite briefly changes that occur in another type of infection. In rabbits inoculated intravenously with *Staphylococcus aureus* the marrow changes were quite in contrast with those found in tuberculous infection. The hyperplasia in staphylococcal infection was, from its onset, predominantly in the neutrophilic series. Because of this the cellular pattern of the marrow did not appear so complex. The differences in the two pictures suggested that the basic demand placed on the marrow was much more unicellular in type in staphylococcal than in tuberculous infection.

Reticulo-endothelial System (*Liver*)

This organ was chosen to represent the so-called reticulo-endothelial system because, in postembryonic life, it is normally free from the other types of hematopoietic tissues. In the normal rabbit liver the reticulo-endothelium (Küpffer cells) can be fairly easily recognized. It is not abundant.

It was found, as is commonly known, that there was a distinct difference in the extent of tuberculous involvement of the liver in bovine and avian tuberculous infection. The avian tubercle bacillus caused much more extensive pathological lesions. The type of cellular response in the individual tuberculous lesion was essentially the same in both types of infection. The accumulations of cells, whether few or many, large or small, occurred almost wholly within the liver capillaries. With the avian tubercle bacillus the greatest involvement was at the period when the animals succumbed, whereas with the bovine type the amount of tuberculosis was usually considerably less when the animals died than at an earlier period.

At 5 days (Fig. 13) the tuberculous lesions consisted of small intracapillary accumulations of mononuclear cells. These lesions were

scattered throughout the organ. The intervening capillaries appeared normal without any evidence of increase or of mitosis in the Küpffer cells. An occasional mitotic figure was found in the tuberculous foci.

At 10 days (Fig. 14) the lesions were more numerous and had the typical appearance of monocytic tubercles. In such foci mitoses were rare. Here again the reticulo-endothelial system away from the focal lesions showed no evidence of hyperplasia.

From 10 days onward in the avian tuberculous infection the volume and number of lesions increased, so that when the animals died at 4 to 5 weeks, large areas composed of capillaries tremendously distended with monocytes were found. The columns of liver parenchyma were compressed between the distended capillaries. At 3 weeks there were many areas of uninvolved liver tissue which showed little, if any, evidence of hyperplasia of the Küpffer cells. In Figure 15 the intracapillary nature of the monocytic tubercle is shown. It is also evident that in the sinusoids adjacent to but separated from the tuberculous lesion there is no evidence of hyperplasia of the reticulo-endothelial system. Some necrosis of monocytes and a beginning invasion of neutrophiles are to be seen in Figure 15. In such lesions tubercle bacilli are abundant.

It is apparent that if the reticulo-endothelial system of the liver does participate in the reaction to the tuberculous infection it does so only in those areas in which the tubercle bacilli lodge. No general hyperplasia of the system such as was found in the myelogenous system had occurred.

In *vaccinated* rabbits reinfected intravenously with avian tubercle bacilli the pathology in the liver differed in some respects from that observed in non-vaccinated animals. The location of the reaction was the same. In the vaccinated animals there often occurred as great a reaction in a week as was found in the non-vaccinated in 2 to 3 weeks. In the vaccinated animals that lived for several months after the introduction of the reinfection a considerable variety of lesions was found. In such animals the major part of the organ was free from infection. The varieties of lesion found were: monocytic tubercles, monocytic tubercles having caseous centers with or without giant cells, isolated foreign body giant cells, small abscesses, foci of lymphocytes, and fibrous scars with or without lymphocytic infiltration. Thus it is seen that vaccination has enabled the animal

to react to the tuberculous infection in a way differing from a first infection.

In the *staphylococcal infection* lesions in the liver were rarely found. Occasional Küpffer cells or neutrophiles might contain the bacteria. When definite accumulations of cells did occur they were predominantly neutrophilic in type.

Lymphoid System (*Spleen*)

The lesions in the spleen are perhaps logically followed by considering the flow of blood through the organ. The arterial system has intimately associated with it closely packed aggregates of small round cells commonly regarded as lymphocytes. These aggregations form a distinctive feature of the architecture of the organ and are spoken of as malpighian corpuscles or germinal centers. The arterial branches pass outward through the germinal centers further to subdivide in the extracorpuscular splenic tissue — the pulp. This portion of the spleen has an exceedingly complex and abundant capillary network lined by endothelium. Between the capillaries is a variable number of round cells which are usually larger than the small ones present in the germinal centers. On the distal side of the pulp the capillaries unite to form the venous sinuses which in turn unite to form branches of the splenic vein. In the venous sinuses the endothelial lining is especially prominent. The terms "germinal center," "pulp," and "sinuses," will be used to designate the locations of the cellular changes observed within the organ.

The germinal center of the normal rabbit spleen (Fig. 18) varies somewhat in size but is always composed of a rather dense mass of small round cells surrounded by a "collar" of larger and paler staining cells. The prominence of the "collar" varies considerably in different animals. The pulp cells resemble those seen in the "collar" of the germinal center. The number of these cells varies considerably. The endothelium of the sinusoids is usually composed of a single layer of flattened cuboidal cells which resemble the cells of the pulp in staining reaction. The remainder of the cellular content of the organ is largely that of the circulating blood.

In tuberculosis the spleen becomes larger than normal (Fig. 16). The degree of enlargement varies considerably. It is especially great in the avian type of infection (Fig. 17) in the rabbit where the organ may become 3 inches long, 1 inch wide and one-half inch thick.

The changes in the spleen following the injection of tubercle bacilli were of the same general character whether the bovine or avian tubercle bacillus was used. Tuberculous involvement was however always much more extensive in the avian type of infection. Within 5 days after inoculation (Fig. 20) hyperplasia of the germinal center had begun, as is evident from the size of the unit and of the "collar." The proportion of the larger cells was increased and mitotic figures were numerous (Fig. 21). There did not seem to be a distinct increase of cells in the pulp or the sinusoids. Mitoses in these latter regions were rare and tubercles were absent. Tubercle bacilli could be demonstrated after careful search in single cells of monocytic type.

Ten days after infection the changes noted above in the germinal center were simply more pronounced (Fig. 22). The small, densely staining type of cell was proportionally reduced. Mitotic figures were numerous (Fig. 23), suggesting a malignant splenic tumor. The cellular content of the pulp was definitely increased and occasional mitotic figures were present. The endothelium of the sinusoids did not appear to have participated to any appreciable extent in the hyperplasia. Among the circulating blood cells megakaryocytes, which in all probability had migrated from the bone marrow, were occasionally seen. An occasional monocytic tubercle was found in the pulp. Tubercle bacilli were easily demonstrable in single cells of monocytic type as well as in the definite tubercles.

The picture seen at 14 days differed from that at 10 days in that typical monocytic tubercles were abundant, some of them being of considerable size. These tubercles were especially prominent at the periphery of the hyperplastic germinal center (Fig. 24). Tubercles were also scattered irregularly through the pulp. It seems as if the large pale staining monocytes of the tubercles had arisen from the cells seen in the "collar" of the germinal center. It was usual to find an admixture of small, deeply staining cells, medium sized, lighter staining cells and the large pale monocytes of the tubercle in the "collar" (Fig. 25). The cellularity of the whole splenic structure was greatly increased. Mitotic figures were found fairly easily, even in monocytes lying free in the blood within the sinuses (Fig. 27). A rare mitotic figure was observed in cells that may have been the endothelial cells of the sinusoids. Tubercle bacilli were abundant, more so in the avian than in the bovine type of infection.

From the 2nd week onward the further changes in the spleen were found chiefly in the tuberculous foci. In the avian type of infection the organ was largely converted into "sheets" of monocytes (Fig. 17, light staining areas) with many of the germinal centers being recognizable only from the presence of the artery and small clumps of small lymphoid cells (Fig. 26). In large areas the monocytes appeared well preserved. There were foci in which these cells had necrosed and in such areas invasion of neutrophiles was frequently found (Fig. 28). In a few areas the neutrophiles had also necrosed, and where this had occurred the typical appearance of caseation was seen. Foreign body giant cells were often abundant in the later stages, especially in the bovine type of infection where the spleen was not so heavily involved. While the splenic tissue remained hyperplastic throughout the course of the disease, mitoses were less easily found at the time of death. Monocytes in mitosis were occasionally found in the tuberculous lesions, and even on rare occasions in giant cells.

In some of the spleens megakaryocytes were quite numerous. There were also the other cells of the myelogenous tissue present. No conclusive evidence could be found that myelogenous tissue was being produced in the spleen.

The reaction that occurred in the spleen in *vaccinated* animals reinfected intravenously with avian tubercle bacilli differed in many respects from that noted in a first infection. Individual tuberculous monocytes and monocytic tubercles were present in all portions of the organ within 5 days and were often more abundant at this early date than at 2 weeks in the animals with a first infection. In the vaccinated animals that survived reinfection for several months the spleens were always found to be within normal size and in some instances appeared smaller than normal. Microscopic examination of such spleens revealed but little evidence of tuberculosis. There were at times small monocytic tubercles or foreign body giant cells in the pulp or in the germinal centers. The germinal centers were consistently smaller and less cellular than normal. No mitotic figures could be found. The cells were largely of the small lymphocytic, with a rare cell of the monocytic type. In the pulp there was evidence of increased reticulum, relatively few pulp cells and considerable amounts of blood pigment in and between the capillaries. In many instances the pigment accumulation was so

large as to distend the capillaries. Pigment-laden monocytes were frequently seen. The endothelial lining of the capillaries and large sinusoids appeared normal. Thus it is seen that through vaccination the animals were enabled to heal, by resolution, the large portion of the tuberculosis of the spleen. It is also quite apparent that with the development of chronic progressive tuberculosis in the kidneys, joints and lungs, the demand for cells of the type manufactured in the spleen became distinctly less than during the earlier stages of the disease. This phenomenon is of real importance since it reflects a mechanism within the different portions of the hematopoietic tissues which responds with delicacy to the demand for cells.

The intense hyperplasia of the splenic tissue and the extensive tuberculous lesions were in great contrast with what happened in *staphylococcal infection*. In the latter the organ was not enlarged if the animals died within a week. Microscopic study showed that there was no evidence of hyperplasia (Fig. 19). If animals lived more than a week the spleen might be slightly enlarged and a suggestion of mild hyperplasia might be found. The contrast between the spleen in tuberculous and in staphylococcal infection suggested a fundamental difference in the demands placed on the organ in the two types of infection. There was also a suggestion that if an animal had sufficient resistance to survive a staphylococcal infection for a considerable time the splenic tissue was called upon, to a mild degree at least, to participate in the disease process.

Lymphoid System (Appendix)

This organ, because of its abundant lymphoid tissue, was chosen to represent the lymphoid system apart from the spleen. In the rabbit the lymph nodes do not seem to participate as much in the tuberculous process as they do in the guinea pig. This may be due in part, but not entirely, to the use of a different method of inoculation. Mesenteric lymph nodes were routinely studied in the rabbits and considerable alteration from normal was found. It was not clear, however, whether the changes noted were due to the reaction of the essential tissue of the nodes or to the accumulation of cells from the lymph channels that drained the lymphoid tissue of the gut. Tuberculous lesions in the nodes were rare unless lesions were also present in the gut.

The unit of lymphoid tissue in the normal appendix (Fig. 29) is somewhat flask shaped. The portion toward the serosa has a rim of closely packed lymphoid cells, while the central portion is composed of a network of blood and lymph capillaries, between which lymphoid cells are closely congregated (Fig. 32). A rare mitotic figure may be found in the peripheral zone. The portion adjacent to the mucosa shows closely packed lymphoid cells which are smaller than those in the serosal region. The amount of lymphoid tissue in normal animals varies considerably but it is seldom more abundant than in the illustration given (Fig. 29).

Within 5 days after the inoculation of tubercle bacilli the lymphoid tissue showed evidence of beginning hyperplasia. This regenerative activity reached its height in 10 to 14 days. Under low power the volume of lymphoid tissue was found to have become greatly increased (Fig. 30), predominantly so in the serosal portion. Under high power (Fig. 33) the latter area showed the peripheral portion definitely thickened and mitotic figures abundant. The central area was also much more cellular than normal and occasional mitoses were found in this region. A study of the area adjacent to the mucosa showed an apparent increase of cells which were smaller and more typically lymphocytes (Fig. 34) than those in the serosal area. Mitotic figures in this portion were rare. As one followed the tissue structure from the mucosa toward the serosa a fairly sharp dividing line occurred between the size of cells and the frequency of mitotic figures.

The intense hyperplasia of the lymphoid tissue of the appendix apparently was not due to the presence of tuberculous infection in the tissue. Tubercles were extremely rare in both the avian and the bovine type of infection when the rabbits died within 6 weeks. The bacilli in isolated cells, so easily demonstrated in other organs, were not found in this tissue.

Tuberculous lesions of the gut do occur when rabbits infected with bovine tubercle bacilli survive for several months. As a rule the longer the animal lives the more numerous are the lesions. The lesions are limited almost wholly to the appendix and the beginning of the cecum. They are due apparently to the ingestion of tuberculous pus from open, ulcerative pulmonary tuberculosis. The foci are irregularly distributed and always occur in the portion of lymphoid tissue adjacent to the serosa (Fig. 31). They show the same sequence

of events that occurs in tuberculous lesions in other tissues. The early tubercle is composed of well preserved monocytes (Fig. 35). When and if the monocytes undergo necrosis such areas are invaded by neutrophiles (Fig. 36) and with the death of the latter cells the typical picture of caseation is produced.

A study of the uninvolved areas of lymphoid tissue in the late stage of tuberculosis was of interest. A comparison of Figures 30 and 31 shows that the serosal portion is less prominent and the mucosal portion is more in evidence in Figure 31. The mucosal area is composed of small typical lymphocytes. The serosal portion is less cellular than normal. Mitotic figures occur in this latter area but they are infrequent.

The reaction of the tissue in *staphylococcal infection* corresponded to that noted under the spleen in the same type of infection. This tissue apparently participated but little in the disease process.

Circulating Blood (*Mobilized Hematopoietic Tissue*)

Wide fluctuations in the cellular content of the circulating blood in rabbits are so common that it is necessary to give much greater latitude for the normal picture than in man. The picture varies so much from rabbit to rabbit that it is advisable to use each animal as its own control. Whether this instability of the blood picture is due to a more labile physiological set-up or to the presence of unrecognized natural infections is difficult to determine. Examination of several hundred apparently healthy rabbits has suggested to the authors that the fluctuations found are more likely to be due to an unstable physiological state than to intercurrent infection.

The cell types are the same in the rabbit and in man, although the staining reaction and the granular content of the different leukocytic types are somewhat different. As a rule the lymphocytic content equals or exceeds the neutrophilic. The lymphocytes commonly outnumber the neutrophiles at least two to one. The monocytic type of cell may, on occasion, be over 10 per cent but it is usually between 3 and 8 per cent. Basophiles (sometimes called the "x" cells) vary from 2 to 10 per cent but on occasion may be more numerous. Eosinophiles show the lowest percentage of any type of leukocyte. Nucleated red cells may be seen on occasion. The total leukocytic count tends to be quite variable.

The technic used in making the total leukocytic counts was that

commonly employed. For the differential counts 400 leukocytes were counted on blood smears stained with Wright's stain. For the modified Schilling count the neutrophiles were divided into seg-

TABLE I

Rabbit No. 8. 1 mg. Avian Tubercle Bacilli Injected Intravenously

Leukocytic record						
Date	Total count	Neutrophiles	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%
4/17/33	21,800	17	68	8	3	4
4/18	12,700	24	62	9	2	3
4/19	19,000	20	67	7	1	5
4/20	17,000	15	78	5	1	1
4/21	12,700	40	50	8	0	2
4/22	10,700	26	60	7	3	4
4/23	11,500	20	63	8	3	6
(4/24	1 mg. avian tubercle bacilli intravenously)					
4/24	12,000	33	59	6	1	1
4/25	8,700	33	54	10	2	1
4/26	14,200	35	45	15	1	4
4/27	6,300	23	67	7	1	2
4/28	8,400	40	34	17	2	7
4/29	10,500	23	58	10	4	5
4/30	5,200	20	66	8	2	4
5/1	7,400	28	61	9	1	1
5/2	6,500	28	57	9	3	3
5/3	9,400	19	67	10	2	2
5/4	8,600	16	76	6	1	1
5/5	15,500	9	61	29	0	1
5/6	18,600	14	40	44	0	2
5/7	13,200	18	52	28	1	1
5/8	31,400	23	37	39	0	1
5/9	14,000	17	21	60	0	2
5/10	9,100	18	54	25	0.5	2.5
5/11	8,900	34	40	25	0.5	0.5
5/12	25,300	15	48	35	1	1
5/13	17,700	17	32	48	0.5	2.5
5/14	17,000	27	30	42	0	1
5/15	17,000	29	25	44	0	2
5/16	6,500	32	34	33	0	1
5/17	7,300	32	23	45	0	0
5/18	15,000	26	20	50	1	3
5/19	14,500	36	22	41	0	1
5/20	5,300	50	22	27	1	0
5/21	7,500	29	18	53	0	0
5/22	13,300	46	30	24	0	0
5/23	11,900	47	21	32	0	0
5/24	13,000	51	20	29	0	0
5/25	Dead					

mented and non-segmented forms. In some of the tables the percentage of immature or non-segmented forms is given. In the immature cells we included all forms that could be definitely identified as belonging to the neutrophilic series and that were not segmented.

TABLE II

Gray Rabbit. 5 mg. Virulent Bovine Tubercle Bacilli Injected Intravenously

Leukocytic record							
Date	Total count	Neutrophiles	Non-segmented neutrophiles 100 cells counted	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%	%
8/22/33	7,000	46	15	43	6	1.5	3.5
8/23	8,400	30	23	61	4	1	4
8/24	6,900	40	22	49	5	0	6
8/25	6,500	47	17	44	4	1	4
8/26	6,800	34	6	58	5	0	3
8/27	9,800	36	16	53	7	0.5	3.5
8/28	11,700	34	20	48	7	2	9
(8/28	5 mg. virulent bovine tubercle bacilli intravenously)						
8/29	12,900	34	29	52	9	0.5	4.5
8/30	10,700	37	30	51	9	0	3
8/31	8,300	40	18	50	7	0.5	2.5
9/2	9,700	53	32	36	9	0	2
9/4	6,500	42	22	54	3	0	1
9/6	8,600	33	42	51	14	0	2
9/7	7,100	21	48	69	8	0	2
9/8	5,800	22	73	71	7	0	0
9/9	6,400	20	59	67	10	0	3
9/10	5,600	19	58	72	7	0	2
9/11	8,300	31	77	51	17	0	1
9/12	9,900	27	70	51	21	0	1
9/13	15,600	41	73	26	32	0	1
9/14	10,600	46	70	32	21	0	1
9/15	7,200	54	75	28	17	0	1
9/16	12,400	75	70	8	17	0	0
9/17	6,900	56	80	29	15	0	0
9/18	Dead						

To show the changes that occur in the leukocytic picture in acute tuberculosis 2 rabbits have been selected for illustration. While the degree of variation from normal may differ considerably in different animals the general trend is always in the same direction. In each of the cases illustrated several leukocytic counts are given before inoculation to indicate what was the "normal" for the animal.

In the case of the rabbit with the avian type of infection (Table I) the ordinary routine leukocytic count is given. It will be noted that

following inoculation the eosinophiles and basophiles gradually drop out of the picture. The balance between the neutrophiles and lymphocytes is not greatly altered for several days, except that it appears as though the neutrophiles tend to recede to or below the lower percentage of normal. Toward the end however the balance is definitely the reverse of normal. From the 10th day on, the monocyte enters the picture definitely. In the tabulation of monocytes we have included in this group all mononuclear cells that evidently did not belong to the other leukocytic types. We believe that among these cells there are many marrow stem cells and young megakaryocytes. Such cells could not, however, be unequivocally differentiated from immature monocytes and they have all been included under the heading of monocytes. An occasional mitotic figure was seen in cells of this type in the blood smears. Thus it is seen that during the evolution of the infection a profound change occurred in the intercellular relation which before death led to a notable increase, proportionally, of neutrophiles and monocytes at the expense of the lymphocytes.

In the case of the rabbit infected with virulent bovine tubercle bacilli (Table II) we have added the immature neutrophiles to the leukocytic count. The percentage in this instance represents that portion of the total neutrophiles which have non-segmented nuclei. This is a modified Schilling count in which stress is placed on the presence in the circulation of immature or non-segmented neutrophiles. A comparison of the interrelation of the leukocytic types before and after inoculation shows the same general trend of events as was seen in the rabbit infected with the avian tubercle bacillus. The difference in the 2 rabbits is only a matter of the degree of change. The addition of the percentage of immature neutrophiles adds an important feature not apparent in the other case. During the period (9–7 to 9–10) when the percentage of neutrophiles has decreased and that of the lymphocytes increased, it is evident that a considerable rise has occurred in the percentage of immature neutrophiles. As the disease progresses and the neutrophile-lymphocyte balance becomes the reverse of normal, the immature neutrophiles continue at a high level. It can readily be seen that in acute tuberculosis in the rabbit it is essential to take into account the immaturity of the neutrophiles, otherwise one would be led to believe that these cells did not enter into the pathological process.

Enumeration of the blood platelets was attempted. It was discontinued because it was felt that with the great variation in the size of the platelets, noticeable as the disease progressed, the number did not give a true indication of the volume of platelet material. From fixed blood smears it was evident that there was at times a considerable increase in the volume of platelets.

Erythrocytic counts were followed in some of the animals, a small proportion of which developed a definite anemia, but this was not a constant feature. A distinct change always occurred in the erythrocytic picture as seen on fixed smears. Nucleated red cells were often numerous. Changes in size, shape and staining reaction of the erythrocytes was always evident toward the end of the infection. Red cells phagocytozed by monocytes were commonly seen in the late stages of the disease.

Since the "systems" of hematopoiesis gave a somewhat different picture in vaccinated reinfected rabbits from that found in the animals with a primary infection, it is of interest to note the change in the circulating blood in the vaccinated reinfected groups. For purpose of illustration an animal vaccinated intravenously with heat-killed bovine type of bacillus and later infected with living organisms of the same strain has been chosen. Animals so treated vary considerably as to the degrees of change in the leukocytic picture, though in general the same trend is noted in all of them. A study of Table III shows the following points of significance: The principal change following the first injection of vaccine was a rise in immature neutrophiles which gradually subsided. Following the second inoculation of vaccine the immature neutrophilic picture again changed and in addition there developed a leukocytosis, a definite increase of monocytes, and a drop in percentage of lymphocytes. All of these returned to a normal status before the injection of the living bacilli. Following the injection of the living bacilli the changes noted above not only recurred but persisted. The fluctuation found in the percentages of immature neutrophiles during the progress of the disease emphasizes the fact that care must be exercised in the interpretation of this phenomenon in infections of long standing. The picture shifted to what may be termed a septic leukocytic picture within a month after the virulent bacilli were injected and so continued to the death of the animal. Toward the end the picture was such as might be found in infections produced by staphylococci and pneumococci.

TABLE III

Black Rabbit. Vaccinated Intravenously with Heat-killed Bovine Tubercle Bacilli. Later 1 mg. Virulent Bovine Tubercle Bacilli Injected Intravenously

Leukocytic record							
Date	Total count	Neutrophiles	Non-segmented neutrophiles 100 cells counted	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%	%
8/22/33	7,700	30	13	60	5	1	4
8/24	11,100	34	15	52	4	3	7
8/26	9,100	38	48	42	5	6	9
8/28	10,600	40	27	48	5	4	3
(8/28	5 mg. heat-killed bacilli intravenously)						
8/29	10,600	38	45	44	10	3	5
8/30	16,100	28	46	60	7	1	4
8/31	6,900	33	28	53	10	1	3
9/2	6,000	34	30	55	6	1	4
9/4	7,400	44	23	47	7	1	1
9/6	8,400	38	34	42	14	1	5
9/9	11,700	47	22	29	11	9	4
9/13	13,900	17	18	62	12	0	9
(9/13	10 mg. heat-killed bacilli intravenously)						
9/14	30,500	60	53	25	8	0	7
9/15	15,400	31	29	39	24	1	5
9/16	18,500	39	35	35	22	0	4
9/17	20,000	40	52	34	20	0	6
9/19	17,000	39	28	42	15	0	4
9/21	21,400	37	19	41	16	3	3
9/24	8,500	37	24	56	6	0	1
9/27	6,300	48	26	42	7	1	2
9/30	7,700	25	28	64	8	0	3
10/4	14,500	25	24	50	23	1	1
10/8	4,500	23	18	67	7	0	3
10/12	7,300	31	26	53	12	2	2
10/15	12,300	37	22	48	11	0.5	3.5
10/19	7,800	31	27	55	7	2	5
10/23	10,600	31	26	62	5	1	1
10/27	7,000	30	14	56	10	0	4
11/1	7,000	18	22	75	5	0.5	1.5
11/3	7,700	34	18	57	6	1	2
11/6	10,500	48	16	34	12	1	5
11/8	19,000	38	18	33	18	0	11
11/10	9,800	35	11	55	5	0	5
11/13	9,000	46	17	46	5	0	3
(11/13	1 mg. living virulent bovine tubercle bacilli intravenously)						
11/14	11,800	47	54	40	6	0	7
11/15	17,300	17	25	65	15	0.5	2.5
11/17	6,600	35	58	48	12	1	4
11/20	17,200	40	45	40	17	0	3
11/22	8,300	23	60	58	12	5	2
11/24	34,000	39	62	36	20	0	5
11/27	9,200	29	62	50	19	0	2

TABLE III (*Continued*)

Leukocytic record							
Date	Total count	Neutrophiles	Non-segmented neutrophiles 100 cells counted	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%	%
11/29/33	6,600	36	70	38	25	0	1
12/1	16,000	27	67	41	31	0	1
12/4	63,300	56	67	17	24	0	3
12/6	23,200	39	67	26	30	0	5
12/8	17,600	74	54	17	7	1	1
12/11	9,200	52	54	35	10	0	3
12/13	9,900	55	51	32	11	1	1
12/15	7,500	48	54	40	10	0	2
12/18	51,000	73	60	12	13	0	2
12/20	7,000	63	60	29	7	0	1
12/22	23,100	58	47	23	18	0	1
12/26	8,300	55	45	35	9	0	1
12/28	12,100	67	61	21	11	0.5	0.5
12/30	18,100	60	37	25	13	0	2
1/2/34	11,300	47	40	26	23	0	4
1/4	9,800	70	31	16	14	0	0
1/8	16,100	56	44	21	20	1	2
1/10	9,500	57	38	25	16	1	1
1/12	6,000	61	32	26	11	2	0
1/15	12,300	50	42	19	29	0	2
1/17	17,700	69	28	14	16	0.5	0.5
1/19	30,000	78	45	7	15	0	0
1/22	12,400	76	34	11	11	0	2
1/24	9,600	69	45	15	15	0	1
1/26	23,400	69	33	13	17	0.5	0.5
1/29	11,600	72	20	13	14	0.5	0.5
1/31	10,900	73	32	16	11	0	0
2/3	34,000	70	26	14	15	0	1
2/5	18,400	71	42	13	15	0	1
2/7	23,000	65	48	10	24	0	1
2/9	18,000	72	55	13	14	0.5	0.5
2/12	23,800	69	40	13	18	0	0
2/15	34,000	68	50	12	20	0	0
2/19	27,100	85	30	3	10	0	2
2/22	16,800	81	50	10	7	0	2
2/24	Dead						

The shifting about of the leukocytic picture indicates not only the delicate interplay of cellular function but it also emphasizes the fact that a single leukocytic count can reveal only the status of the pathological process at the time the count is taken. Thus leukocytic counts taken at different times may reflect the different phases of a pathological process within the body.

Thus it will be seen that the circulating blood gives definite evidence of a profound reaction in the hematopoietic tissues as the disease progresses. In all of the animals the shift in the circulating blood picture lagged behind the changes found in the quasi-fixed portions of the system.

To complete the brief comparison we have been making between staphylococcal and tuberculous infection the leukocytic record of 2 rabbits given an intravenous injection of *Staphylococcus aureus* are appended. The 2 rabbits selected for illustration were given the same dosage of cocci on the same date. One (Table IV) died within 5, and the other (Table V) within 17 days.

TABLE IV

Rabbit No. 1. 1 mg. Staphylococci Injected Intravenously

Leukocytic record							
Date	Total count	Neutrophiles	Immature neutrophiles 100 cells counted	Lymphocytes	Mononuclears	Eosinophiles	Basophiles
		%	%	%	%	%	%
1/24/33	3,900	52	15	34	8	2	4
1/25	8,000	59	16	28	7	2	4
1/26	6,500	60	20	26	8	2	4
1/27	8,600	28	18	59	9	0.5	3.5
1/29	5,500	58	13	33	5	1	3
1/30	2,500	50	20	33	7	1	9
(1/31 Staphylococci injected)							
1/31	5,100	40	19	41	9	1	9
2/1	8,900	89	41	5	3	1	2
2/2	17,200	68	33	4	12	0	16
2/3	16,600	81	44	5	8	1	5
2/4	2,600	73	63	8	10	1	8
2/5	Dead						

Tables I to V record the variations observed in the circulating leukocytic picture in different types of infection. The differences observed in animals given the same type of infection are given. Also the effect of changes in the tissues through vaccination on the leukocytic response is depicted. A careful study of the leukocytic records reveals a considerable variation in the interplay between the various leukocytic types both prior to and after the introduction of the infectious agent. The degree of change is much greater after the injection of the bacteria.

Correlation of the Cellular Interplay

The changes that have occurred in the hematopoietic tissue have been presented under the headings of "systems" so that it would be possible to describe the successive alterations that occurred in each "system." To correlate the findings in the hematopoietic tissue as a whole we now take a single animal (Table II) and show what has

TABLE V

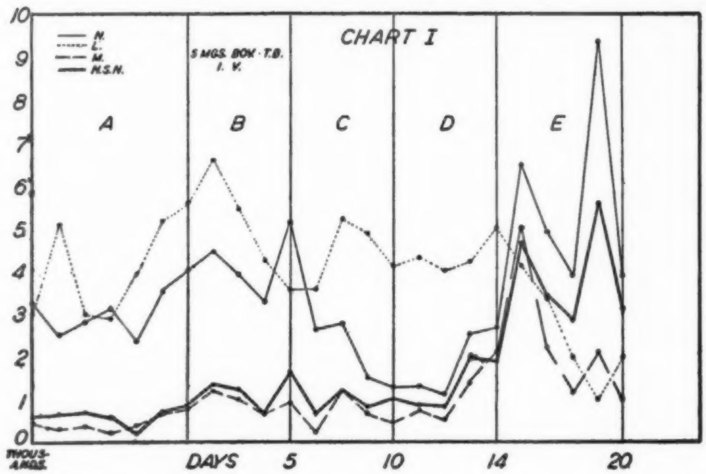
Rabbit No. 3. 1 mg. Staphylococci Injected Intravenously

Leukocytic record							
Date	Total count	Neutrophiles	Non-segmented neutrophiles 100 cells counted	Lymphocytes	Mononuclears	Eosinophiles	Basophiles
		%	%	%	%	%	%
1/24/33	9,700	42	23	47	9	1	1
1/25	9,700	37	21	56	5	1	1
1/26	11,700	36	27	53	9	..	2
1/27	12,700	42	23	52	5	..	1
1/29	9,700	32	12	60	6	..	2
1/30	12,200	33	12	61	4	1	1
(1/31	Staphylococci inoculated intravenously)						
1/31	9,700	43	16	52	3	1	1
2/1	12,500	74	51	16	5	..	5
2/2	26,100	45	44	15	30	..	10
2/3	25,400	53	49	21	19	0.5	6.5
2/4	31,700	50	54	16	18	..	16
2/5	20,400	42	57	11	21	..	26
2/6	43,700	39	56	16	29	..	16
2/7	33,000	62	55	8	24	..	6
2/8	39,600	59	52	14	23	..	4
2/9	21,200	63	52	11	21	..	5
2/10	41,000	67	57	12	15	..	6
2/11	36,000	76	52	8	15	..	1
2/12	113,000	86	55	3	11	..	..
2/13	39,300	76	40	7	15	..	2
2/14	43,000	81	59	4	12	..	3
2/15	100,800	89	73	4	7	..	..
2/16	20,600	84	76	6	9	..	1
2/17	Dead						

been the hematopoietic response during the course of the disease. In Chart 1 graphs of the total number of the different leukocytic types known to participate in the tuberculous lesions are given. It will be noted that with the progress of the infection a marked alteration occurs in the interrelation of the different cell types. We have divided (A, B, C, D and E) the story told by the circulating leuko-

cytes to correspond to the periods of observation of the quasi-fixed portion of the hematopoietic tissue.

In Table VI the picture of the hematopoietic tissue as a whole is given. In the first part of the table the proportional relations of the different leukocytic types shown in Chart 1 are given, using the monocyte as the basal unit. Here we have used the average for all observations made during each division (A, B, C, D and E) in the chart. In the second portion of the table a composite picture of the condition of each "system" is given for each division of the chart. From the table it is evident that it takes several days for the blood to



"mirror" the changes occurring in the quasi-fixed portion of the blood-forming tissues. Thus the changes in the circulating blood in C reflect the changes in the spleen, marrow and lymphoid tissue in B, and so on. It has been our experience that this lag continues and that evidence of lessened hyperplasia occurs in the hematopoietic tissues before such a phenomenon is observed in the circulating blood.

Chart 1 and Table VI clearly show that the hematopoietic tissue as a whole is involved in the response to the tuberculous infection. As the disease progresses, shifts in the degree of response of the different "systems" occur and, allowing for a lag, these shifts become evident in the circulating blood. Thus the hematopoietic response to tuber-

TABLE VI

A Correlation of the Changes Occurring in the Different Portions of the Hematopoietic Tissue During the Progress of Tuberculous Infection

Division of Chart 1	Proportional relations of circulating leukocytes				Bone marrow	Spleen	Lymphoid tissue of appendix	Reticulo-endothelium of liver
	Neutrophiles	Non-segmented neutrophiles	Lymphocytes	Monocytes				
A	6.79	1.20	9.11	1.00	Normal Fig. 1.	Normal Figs. 16 & 18	Normal Figs. 29 & 32	*Normal*
B	4.51	1.27	5.32	1.00	Early hyperplasia. Fig. 2. Scattered cells containing tubercle bacilli	Early hyperplasia. Figs. 20 & 21. Rare tubercle	Early hyperplasia. No tubercles	*Normal.* Early tubercle formation. Fig. 13
C	3.46	1.46	7.37	1.00	Marked hyperplasia. Fig. 3. Rare tubercle	Marked hyperplasia. Figs. 22 & 23. A few tubercles	Marked hyperplasia. No tubercles	*Normal.* Numerous tubercles. Fig. 14
D	1.67	1.17	3.80	1.00	Marked hyperplasia. Figs. 4, 5 & 6. A few tubercles	Marked hyperplasia. Figs. 24 & 25. Tubercles numerous	Marked hyperplasia. Figs. 30, 33 & 34. No tubercles	*Normal.* Tuberculosis more extensive
E	2.47	1.71	1.08	1.00	Marked hyperplasia. Figs. 7, 8 & 9. Extensive tuberculosis	Hyperplasia not so marked. Figs. 17, 26, 27 & 28. Tuberculosis extensive	Hyperplasia less evident. A rare tubercle	*Normal.* Extensive tuberculosis. Fig. 15

culous infection is not with one cell type but rather with an interplay of several cell types.

COMMENT

It is the purpose of this paper to demonstrate that the introduction of virulent tubercle bacilli into the animal body creates a complex condition that demands an interplay of various cell types of the hematopoietic tissue to counteract the damage produced. That is, the pathology of tuberculosis is not a one cell type of pathology. It is evident that the cells that constitute tuberculous lesions come in large part from the hematopoietic tissue. The changes that occur in the quasi-fixed areas of hematopoiesis are then of fundamental importance.

Since different cell types of the blood are involved it is necessary to consider the hematopoietic tissue as a whole. Due regard must be given to the widespread distribution of the specialized "systems" that comprise the tissue, to the inherent complexity of each "system" because of the pluropotentiality of the primitive or "stem" cells, to the essential mobility of the tissue, and to the various cell types that are components of the mobilized portion (the circulating blood) of the tissue.

The data in the text demonstrate clearly that the marrow, the spleen and the lymphoid tissue respond early and intensely to the infection. The "reticulo-endothelial system," if one may judge from the reaction in the liver, plays little if any part. The fundamental response in the "systems" reverts early to the primitive blood cells. This renders an interpretation of the hematopoietic response difficult because at present there are no technical methods that make possible the positive identification, or certain the maturation process, of any one primitive cell. A great deal of the confusion that now exists in the terminology of hematology rests here. Too great zeal to "tag" primitive blood cells should be discouraged until such time as positive identification can replace theoretical concepts.

As time elapsed it became evident that the principal demands were for neutrophiles and megakaryocytes from the bone marrow, and for monocytes from the spleen and lymphoid tissue. There was also evidence in some animals that increased erythrocytic production from the bone marrow was required. For some time the production of lymphocytes did not seem to be affected. Later in the infection

the evidence pointed to a continued demand for neutrophiles with a lessening of the need for monocytes, lymphocytes and megakaryocytes. This same sequence of events was found to occur in animals that had been partially immunized by vaccination, with the exception that the hematopoietic response was more rapid than in non-vaccinated rabbits.

There is a general belief that the myelogenous portion of the hematopoietic tissue does not play a prominent rôle in the body's reaction to pure tuberculous infection. Whenever this tissue has been found to participate, the reaction has been attributed to the presence of secondary invaders, such as streptococci, pneumococci, and so on. Cunningham *et al.*,[2] have noted that in experimental animals the bone marrow is often found to be hyperplastic in the later stages of tuberculosis. They have attributed this condition to an over-regeneration following a depression of marrow function caused by the presence of tuberculous infection in the tissue. Our observations are that marrow hyperplasia begins shortly after the inoculation of tubercle bacilli and before definite tubercles are present. This hyperplasia continues until the death of the animal even if the marrow becomes the site of extensive tubercle formation. We have not obtained any evidence suggestive of a depression of hematopoietic activity in this tissue.

The intense response found in the spleen and lymphoid tissue of the vermiform appendix suggest that in acute tuberculous infection these tissues are concerned primarily with the production of monocytes. It appears that the primitive cells of this portion of the hematopoietic apparatus differentiate into monocytes. Whether or not the primitive cells of lymph nodes can also mature in this same direction we cannot state with certainty. We found no evidence that the monocyte of the rabbit is produced in the marrow and liver or from "fixed tissue cells." Our findings correspond closely to those of Bloom.[3] We do not believe, as Bloom does, that the differentiated lymphocytes can become transformed into monocytes but we do believe that the primitive cell may be the same for the lymphocyte and the monocyte. Witts and Webb[4] regard the monocyte as originating from the marrow tissue as well as from the spleen. They state that the monocytes of the marrow are "less mature than in the spleen." We wonder if the monocyte can be correctly identified in such an immature state as the one they observed in marrow preparations

and if they may not have been dealing with the very early stages of other marrow cell types, perhaps the megakaryocyte. Naegeli,[5] and others, maintain that the monocyte originates in the myelogenous tissue. Until it becomes possible to identify unequivocally the stem cell, from which the monocyte develops, differences of opinion will remain as to whether or not this cell type really does have a myelogenous origin. Our data are against such an origin in the rabbit infected with the tubercle bacillus.

Evans, Bowman and Winternitz,[6] Aschoff,[1] Cunningham *et al.*,[2] Foot,[7] Permar,[8] and others, regard the monocyte as arising from the "reticulo-endothelial system." From our studies we have become skeptical as to the existence of such a "system." The essential mobility of the blood tissue and the fact that immature and even primitive blood cells, which retain their propagating propensities, enter the circulating blood, render the presence of mitotic figures and the location of blood cells in sections of tissue of doubtful significance relative to the origin of such cells.

The changes we have found in the circulating blood picture correspond closely to those reported by Cunningham *et al.*,[2] and others. One point which the various reports have not stressed, and which we believe to be significant, is the definite increase in the proportion of immature neutrophiles as the tuberculosis progresses. This phenomenon we have found to be extremely pronounced, even when a severe neutropenia existed. Failure to recognize the occurrence of this great "shift" in the neutrophilic picture has probably been largely responsible for the dictum that neutrophiles do not play a significant rôle in pure tuberculous infection. In previous communications[9, 10, 11] attention has been called to the important part played by the neutrophile in certain phases of the pathogenesis of tuberculosis. The presence of the definite "shift to the left" of the neutrophilic picture in the circulating blood further emphasizes the observations we have previously reported.

From our blood studies on tuberculous animals that have lived for several months we have found that there is a distinct tendency for the proportion of immature neutrophiles to decrease after the 1st month, even though the animals eventually died of the infection. On occasion we have observed that the proportion of non-segmented neutrophiles had returned to normal, although the total number of neutrophiles was well above normal. We believe this to be due

to the fact that the myelogenous tissue had expanded sufficiently so that neutrophiles could be matured in large enough numbers to meet the demand. This would indicate that a decrease in the immature neutrophiles in the circulating blood does not necessarily signify an improvement in the pathological process. This emphasizes the fact that the leukocytic picture as a whole, rather than any one portion of it, should always be considered.

We have found it difficult to make accurate differential leukocytic counts as the acute tuberculous infection progressed, since cells so immature as to render identification mere guesswork entered the circulation. In the tabulations we have included these immature cells with the monocytes. We believe that the large majority of such cells were either undeveloped megakaryocytes or monocytes. Such cells make up from 5 per cent to 20 per cent of the cells classed as monocytes.

As mentioned in the text, changes in the circulating blood lag behind those in the quasi-fixed hematopoietic tissues. This is especially true after the available supply of matured cells is used up during the first 3 or 4 days following the introduction of the tubercle bacilli. Although a lag occurs, the circulating blood in time "mirrors" the changes that have occurred in the bone marrow, spleen and lymphoid tissue.

During the evolution of a pathological process conditions are not static. At one stage the picture found may bear little resemblance to that of another period. This is especially true in circumstances where the hematopoietic tissue is involved, as is shown by the data presented in the text. Not only does the appearance of the different portions of the hematopoietic system vary from time to time in a single type of infection, but it differs even more in different kinds of infection. There is, as well, a considerable variation among individuals with the same type of disease which must be taken into account. Since these conditions exist, the picture found on the examination of a blood smear or of a section of marrow or lymph node should be interpreted as representative of a certain phase of a pathological process rather than as diagnostic of the whole process.

The cellular complexity, together with numerous maturation processes, makes it practically impossible to give a simple and coordinated description of the changes that have occurred in the whole hematopoietic tissue during the evolution of lethal tuberculosis.

We have purposely avoided the coining of new names and have attempted to remain within the realm of clearly demonstrated facts. The creation of elaborate classification, the coining of new names to label cells at frequent intervals during the maturation process and at different stages of functional activity, and the dividing of the hematopoietic tissue into many "systems" we believe simply adds confusion to the understanding of a tissue that nature has left in a topsy-turvy state — perhaps for a reason.

Regardless of the fundamental complexity of hematopoiesis, the important part played by the blood-forming tissue in many and diverse pathological conditions requires that greater effort be made to coördinate the changes that occur. The data we have presented in the text show beyond question that in serious tuberculous infection all parts of the hematopoietic apparatus are affected in one way or another. Also we have shown that a very definite interplay of the different cell types occurs during the progression of the infection. We believe that in all studies where the hematopoietic tissues are under consideration this tissue should be investigated as a whole rather than to examine one or another "system" and ignore the rest. Such investigations should always include careful studies of the circulating blood. One fact that remains paramount is that the blood is in essence a mobile tissue and that the "fixed tissue" portion is only quasi-fixed — factories from which the circulating blood is replenished.

Summary and Conclusions

From our studies of the complex changes and of the interplay of different cell types in the hematopoietic tissue during the progression of acute tuberculous infection in rabbits we can briefly summarize the conditions found as follows:

1. All systems of the hematopoietic tissue are involved.
2. The stem cells are greatly increased.
3. Undifferentiated cells, perhaps stem cells, enter the circulation. These cells retain the ability further to multiply within the circulating blood and also in all probability after entering tissues other than blood-forming. This adds greatly to the uncertainty as to whether a cell in mitosis has arisen *in situ* or in some far removed portion of the hematopoietic tissue.
4. The cells in the germinal centers of the spleen are probably

stem cells which, in acute tuberculosis in the rabbit, differentiate into monocytes. It also seems probable that the pulp cells of the spleen have the same origin.

5. It seems probable that monocytes also originate from at least some portions of the lymphoid tissue outside the spleen.

6. No evidence was found to suggest that the monocyte arises from the marrow or from the reticulo-endothelial system outside of the lymphoid system.

7. The criteria on which the concept of a reticulo-endothelial system rests are insufficient to establish such a system.

8. The circulating blood mirrors the changes found in the so-called fixed hematopoietic tissues, although there may be a lag of several days before changes are reflected.

9. The interplay of various cell types of the hematopoietic tissue necessitates a consideration of this tissue as a whole in pathological processes where it may play an important rôle. Single observations should be regarded as indicative of a phase in a pathological process rather than as characteristic of the process as a whole.

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DESCRIPTION OF PLATES

In all of these experiments tubercle bacilli were inoculated intravenously.

Plate 133

Fig 1. Central portion of femur marrow of a normal rabbit. This portion of the marrow is normally composed in large part of fat cells. Hematopoietic tissue in this area is made up largely of erythrogenic tissue with a scattering of other cell types. × 500.

Fig. 2. Central portion of femur marrow 5 days after inoculation of avian tubercle bacilli. There is an increase of hematopoietic tissue. Myelocytes, megakaryocytes and stem cells definitely increased. No tubercles found. Occasional mitotic figures are present. × 300.

Fig. 3. Central portion of femur marrow 10 days after injection of avian tubercle bacilli. Tissue intensely hyperplastic. Mitoses abundant — three present in illustration. Cells predominantly of the stem cell variety. Rare small monocytic tubercles are present at this stage. × 800.

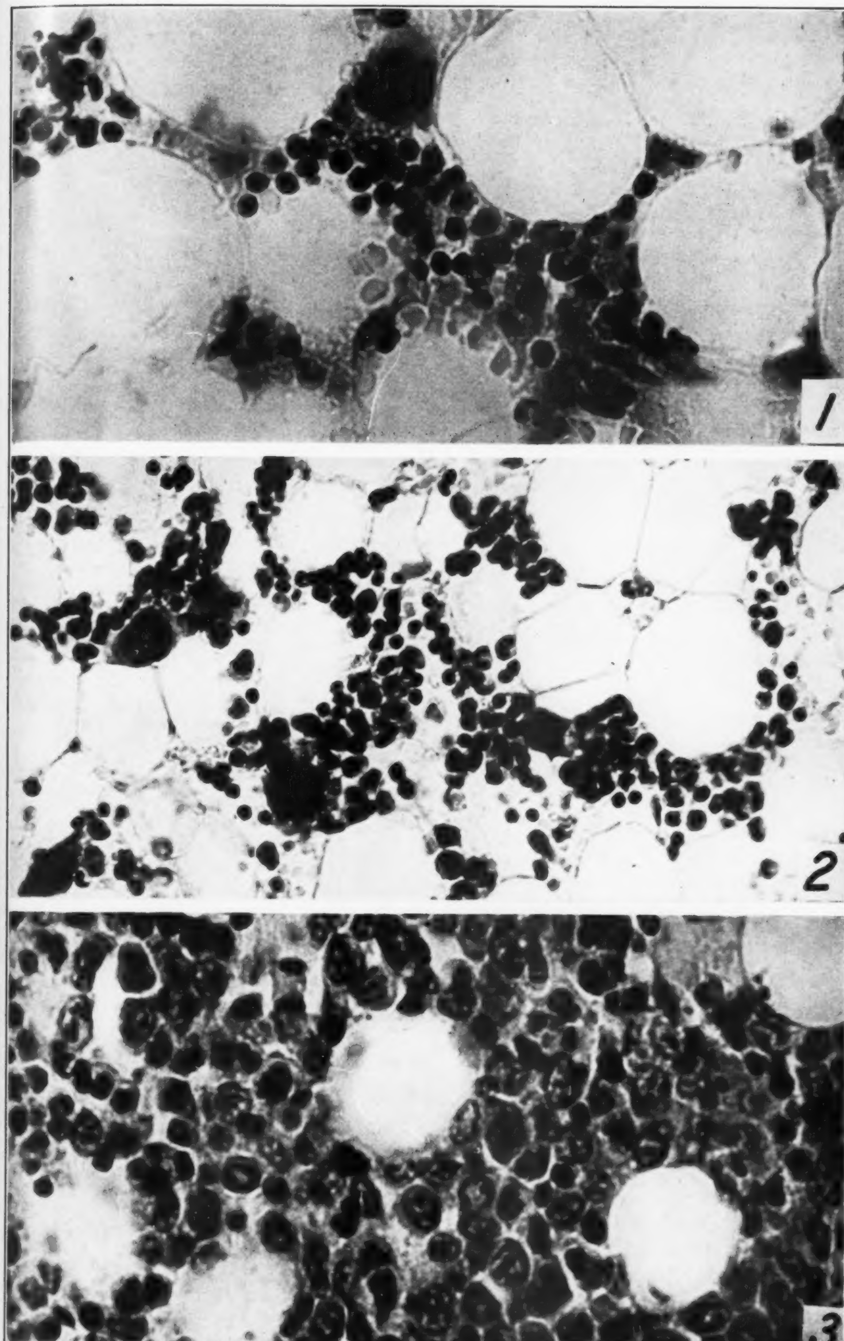
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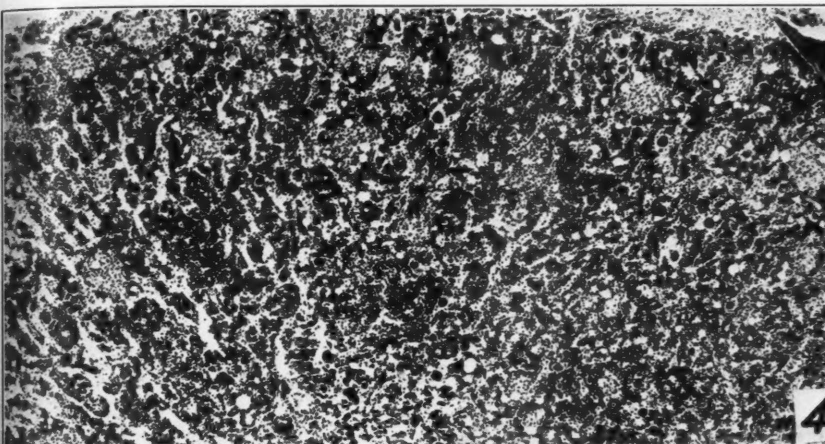
Plate 134

Fig. 4. Femur marrow 14 days after injection of avian tubercle bacilli. Extensive hyperplasia throughout marrow. There is now no essential difference between cellular content of the peripheral and central portions of the marrow. Fat cells widely separated (appear as clear circles in illustration). Note scattered, well defined tubercles which are largely in the peripheral portion of the marrow. × 100.

Fig. 5. High power from Fig. 4. An area in central portion of marrow giving a picture quite similar to Fig. 3. Note megakaryocyte emigrating into blood capillary. × 800.

Fig. 6. High power of a tubercle in Fig. 4. Note the contrast in staining reaction of the tuberculous monocytes and the stem cells in Fig. 5. There is a mitosis in a tuberculous monocyte in the upper right hand quadrant. × 800.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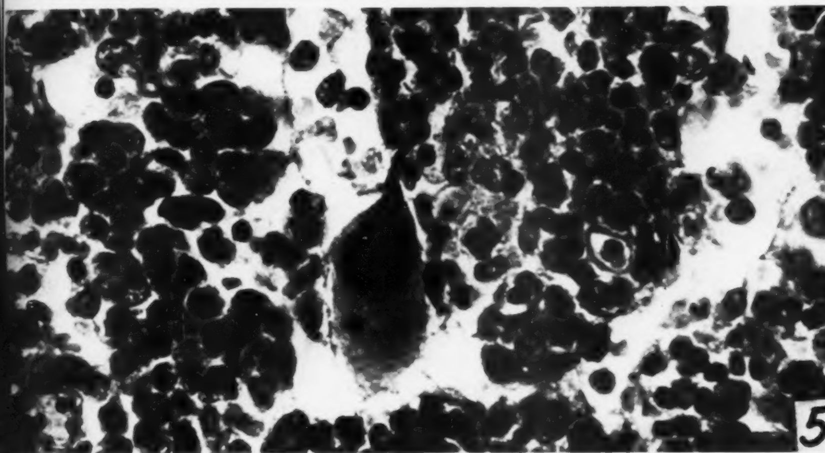
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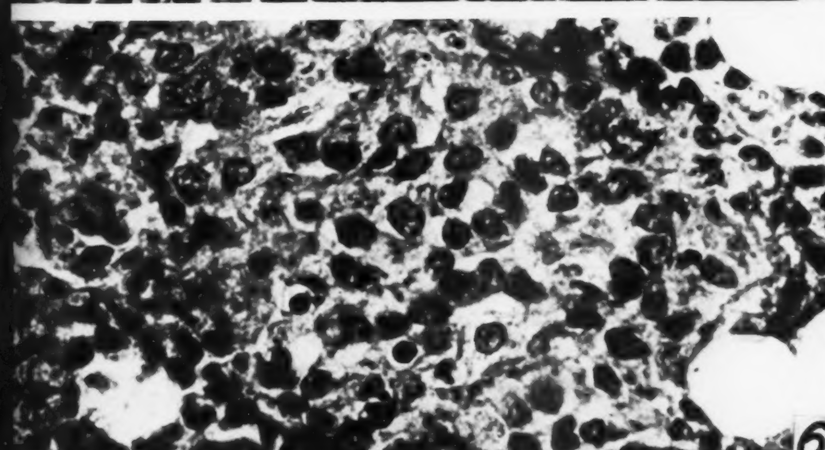
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PLATE 135

FIG. 7. Femur marrow 3 weeks after inoculation of avian tubercle bacillus. Note uninvolved hyperplastic marrow at the periphery (upper part of illustration) and the extensive sheets of tuberculous tissue. × 100.

FIG. 8. From upper portion of Fig. 7. Note the hyperplastic marrow tissue composed largely of stem cells in the upper portion and the sheet of well preserved tuberculous monocytes in the lower portion. × 500.

FIG. 9. From lower portion of Fig. 7. Note the necrosis of the monocytes and a mild infiltration of neutrophiles (the irregular deeply staining masses are the nuclei of neutrophiles). × 800.

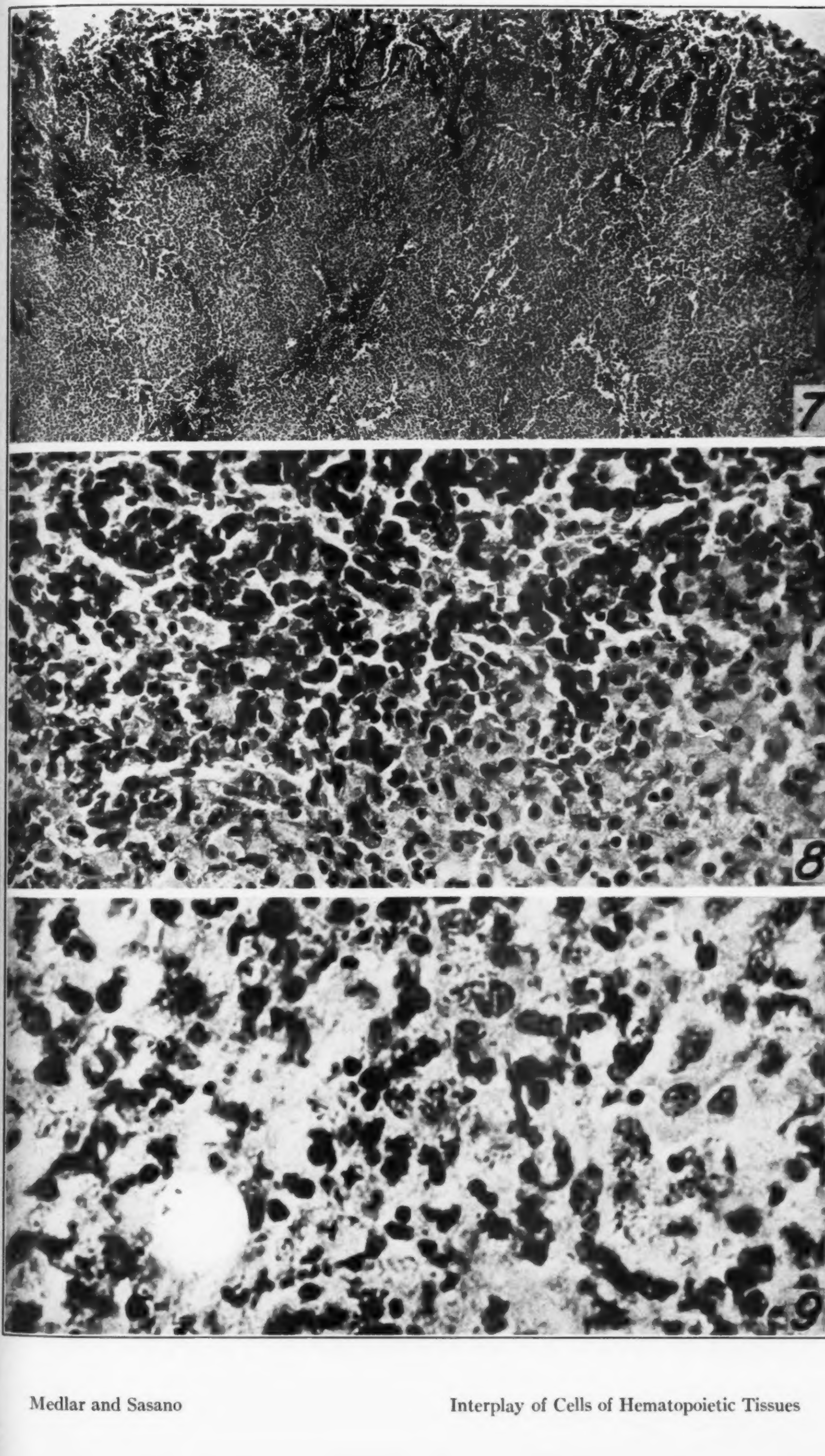
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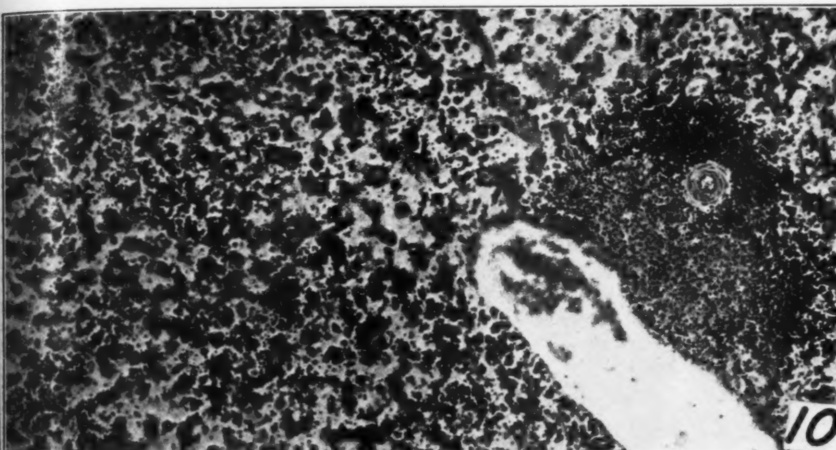
Plate 136

Fig. 10. Marrow from a rabbit vaccinated with heat-killed avian tubercle bacilli and later infected with living organisms of the same strain. Animal died 4 months after the injection of the living bacilli. Marrow is devoid of fat and congested. Note the tuberculous lesion adjacent to the large vessel. This lesion is composed of a mixture of monocytes and lymphocytes with a heavy infiltration of lymphocytes about the periphery. × 100.

Fig. 11. An area from Fig. 10. While the marrow is not crowded with cells the hematopoietic tissue present is hyperplastic. There are six mitotic figures in this field, two of which are in focus. Cells are largely of the myelocytic and neutrophilic types. × 500.

Fig. 12. Marrow from an animal infected with 0.01 mg. of highly virulent bovine type of tubercle bacilli. Animal died within 3 months. Note the large tuberculous lesions, the upper one having a caseous center. The uninvolved marrow is similar to that in Fig. 10. × 100.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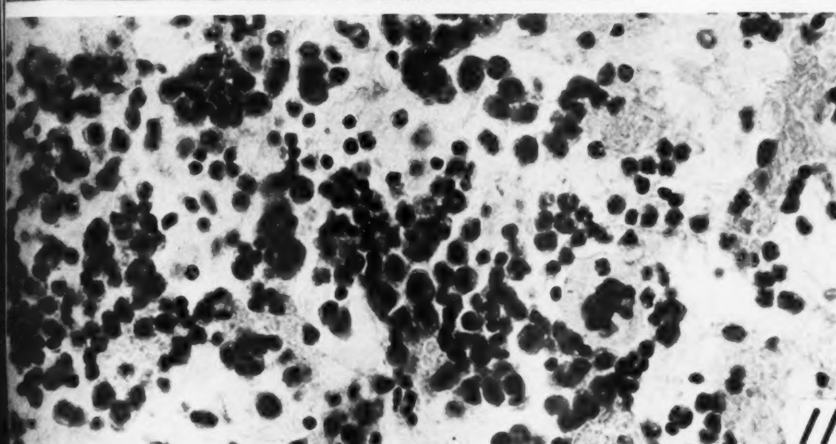
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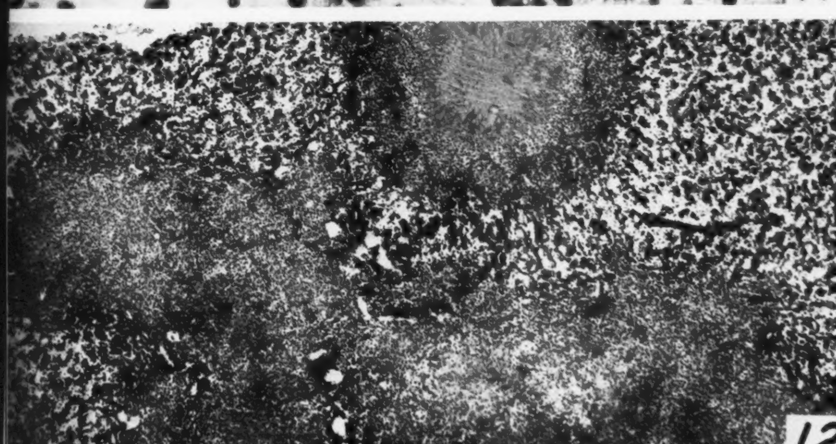
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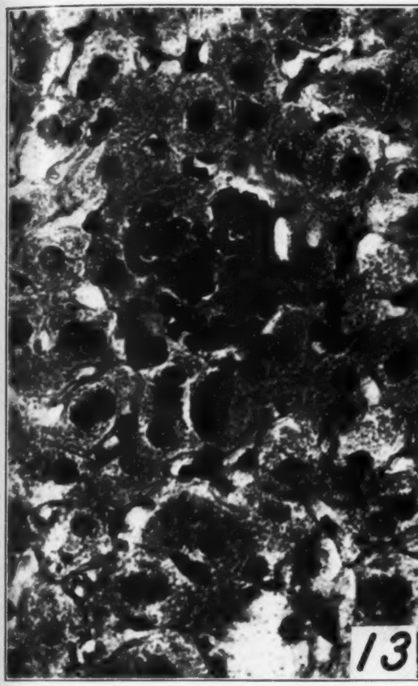
PLATE 137

FIG. 13. Liver 5 days after inoculation of avian tubercle bacilli. Tuberculous lesion is composed of a small intracapillary accumulation of mononuclear cells (upper center). Note that there is no evidence of increase of Küpffer cells outside of tuberculous lesion. $\times$ 800.

FIG. 14. Liver 10 days after inoculation of avian tubercle bacilli. Note typical monocytic tubercle in central portion. This lesion is larger than in Fig. 13 but the tissue is otherwise similar to it. $\times$ 800.

FIG. 15. Liver 3 weeks after inoculation of avian tubercle bacilli. Note large intracapillary lesion which shows some necrosis of monocytes and beginning infiltration of neutrophiles. Note that there is no evidence of increase of Küpffer cells in capillaries adjacent to tuberculous focus. $\times$ 800.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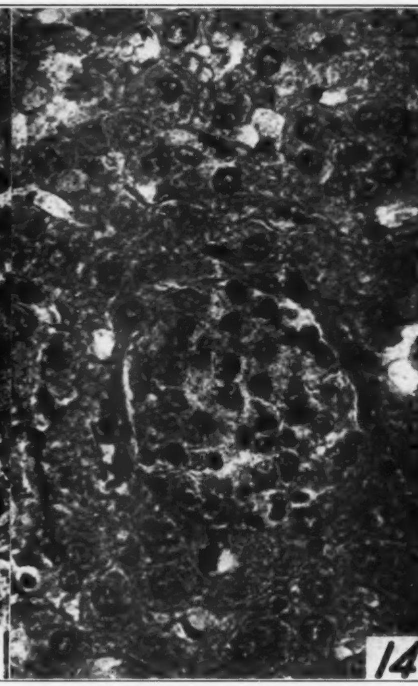
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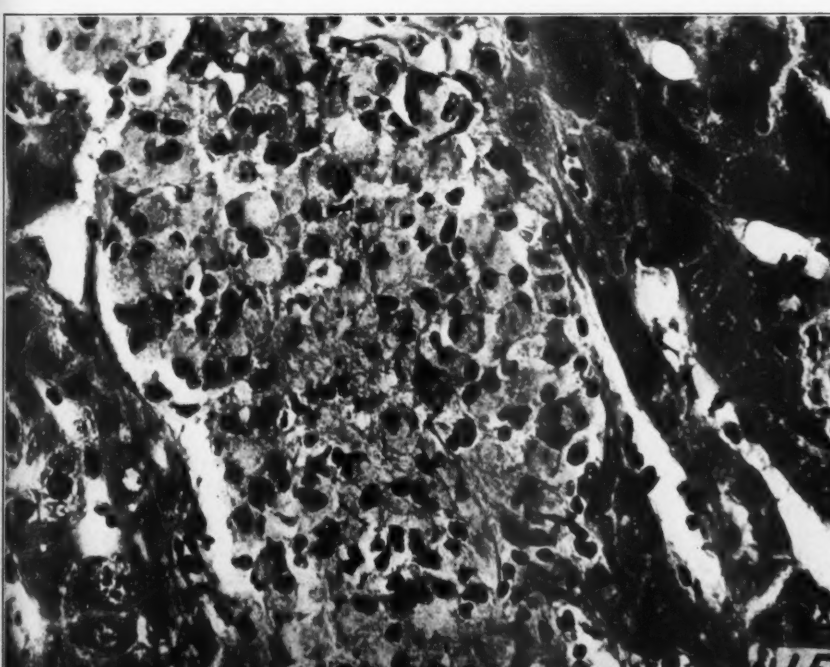
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Plate 138

Fig. 16. Normal rabbit spleen. × 10.

Fig. 17. Spleen 3 weeks after inoculation of avian tubercle bacilli. Organ is composed in large part of monocytic reaction to tubercle bacilli. × 10.

Fig. 18. Germinal center from Fig. 16. Note the closely packed, small, deeply staining cells in the central portion and the peripheral "collar" of larger, lighter staining cells. Note similarity of cells in pulp and those in the "collar." × 200.

Fig. 19. Germinal center in spleen of rabbit that died 5 days after the inoculation of *Staphylococcus aureus*. No evidence of hyperplasia. Compare with Figs. 18, 20, 22 and 24. × 200.

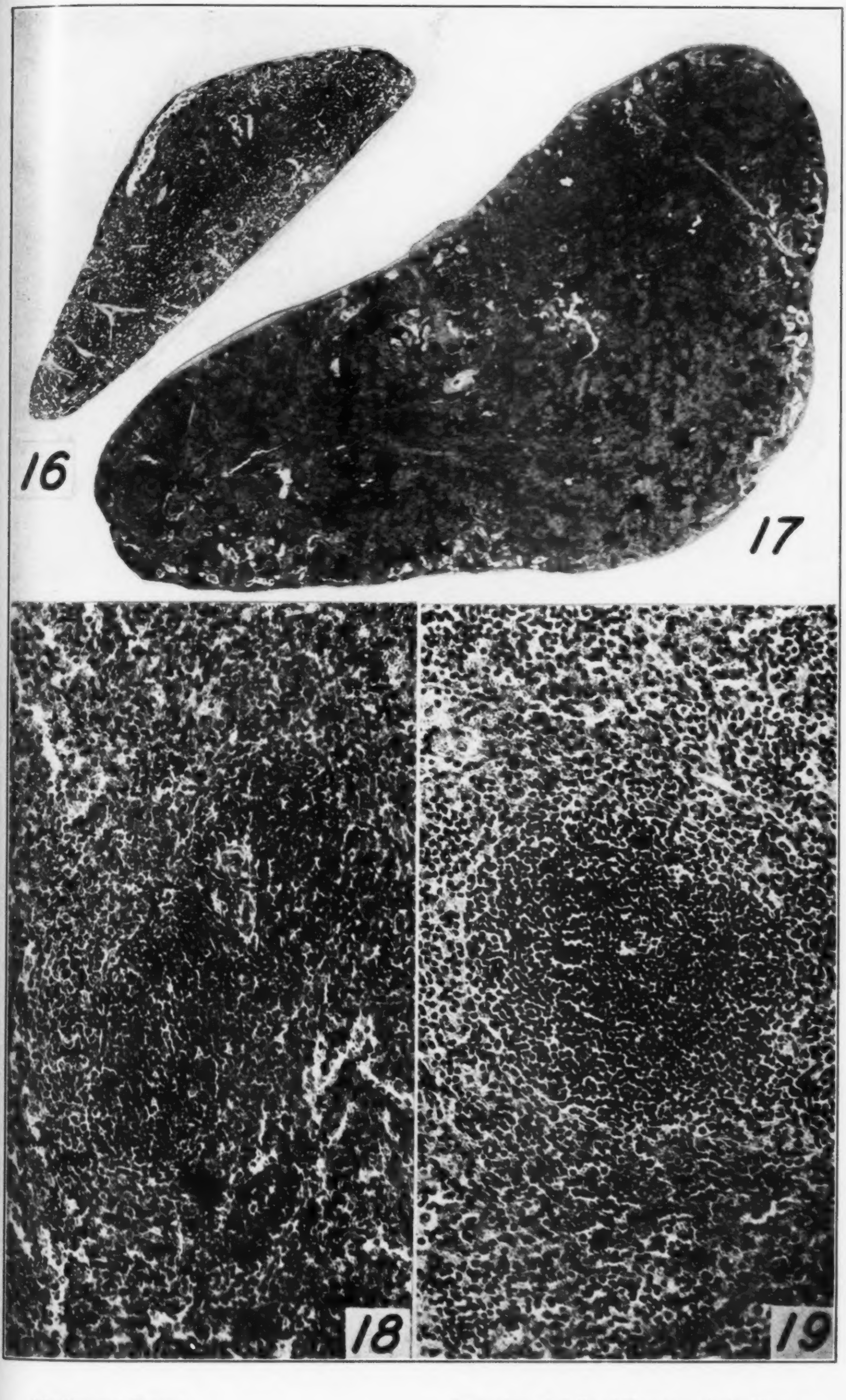
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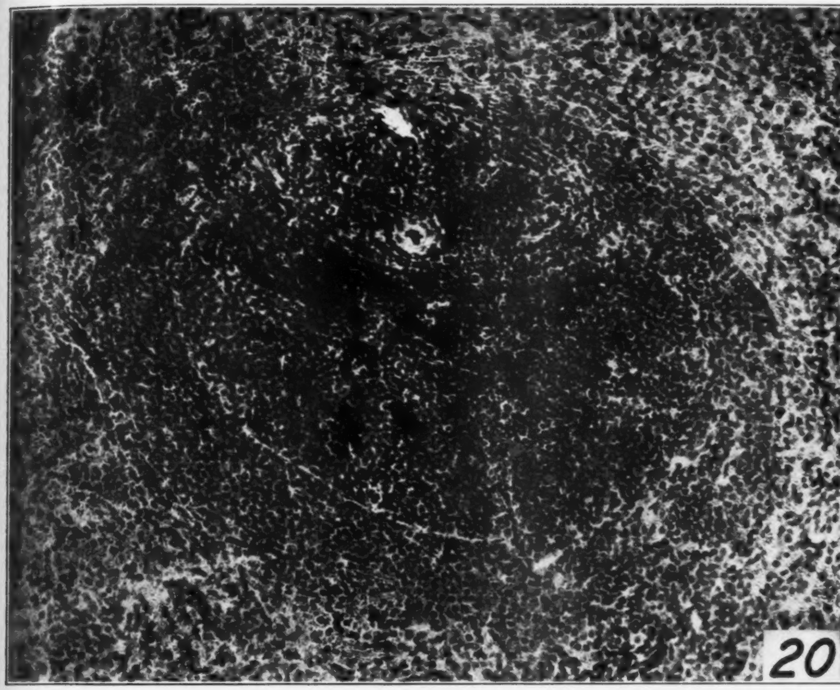
Plate 139

Fig. 20. Germinal center of spleen 5 days after inoculation of bovine type of tubercle bacilli. Note that the germinal center is enlarged, the "collar" is prominent and the pulp is no more cellular than normal. Compare with Figs. 18 and 29. × 200.

Fig. 21. Higher power of Fig. 20. Note two types of cells — small deeply staining, and larger paler staining. Mitotic figures numerous. No tubercles. × 400.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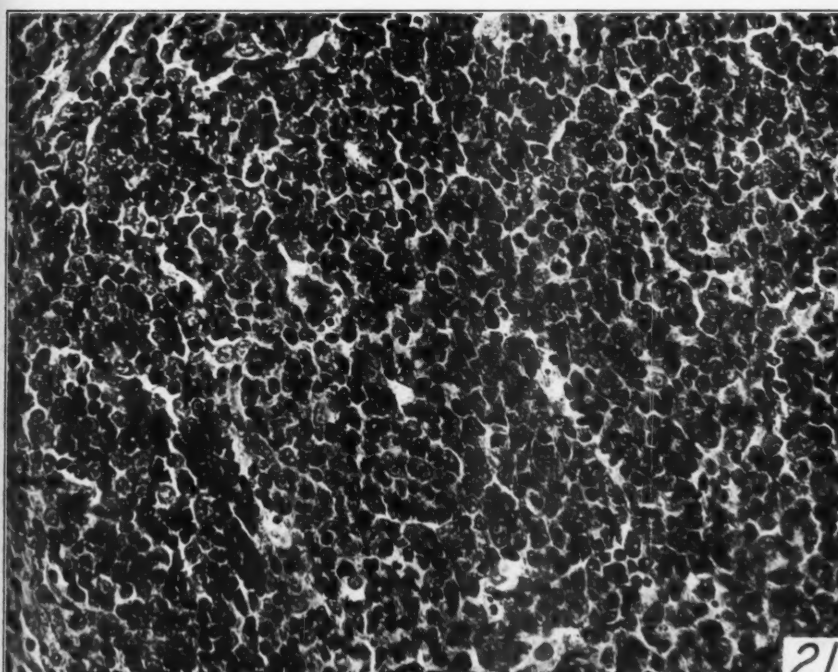
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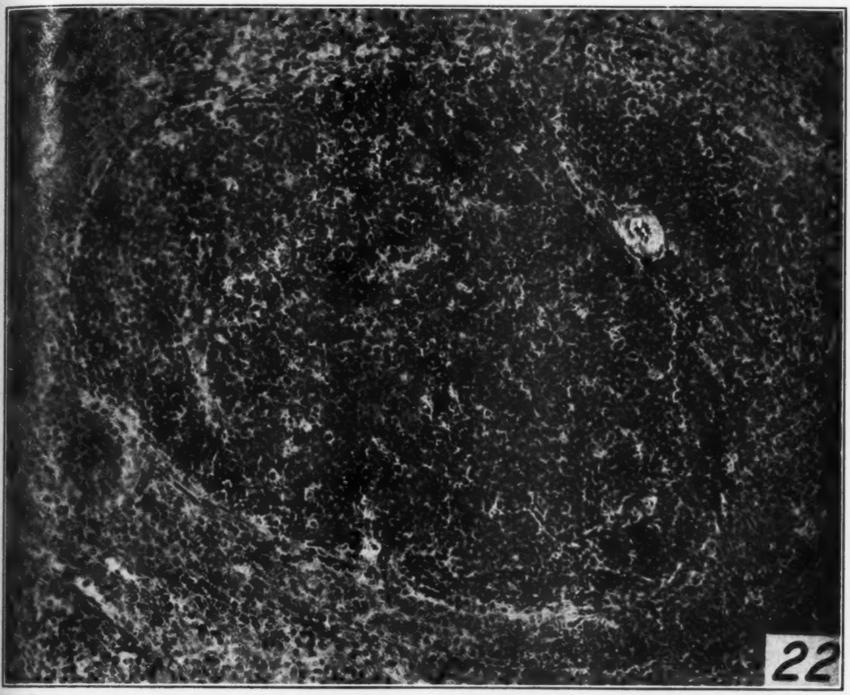
PLATE 140

FIG. 22. Germinal center of spleen 10 days after inoculation of avian type of tubercle bacillus. Note greater hyperplasia than in Fig. 20. Pulp more cellular. × 200.

FIG. 23. Higher power of Fig. 22. Note that cells are in large part composed of the larger, paler staining type of cell. Mitoses numerous. Compare with Fig. 21. × 400.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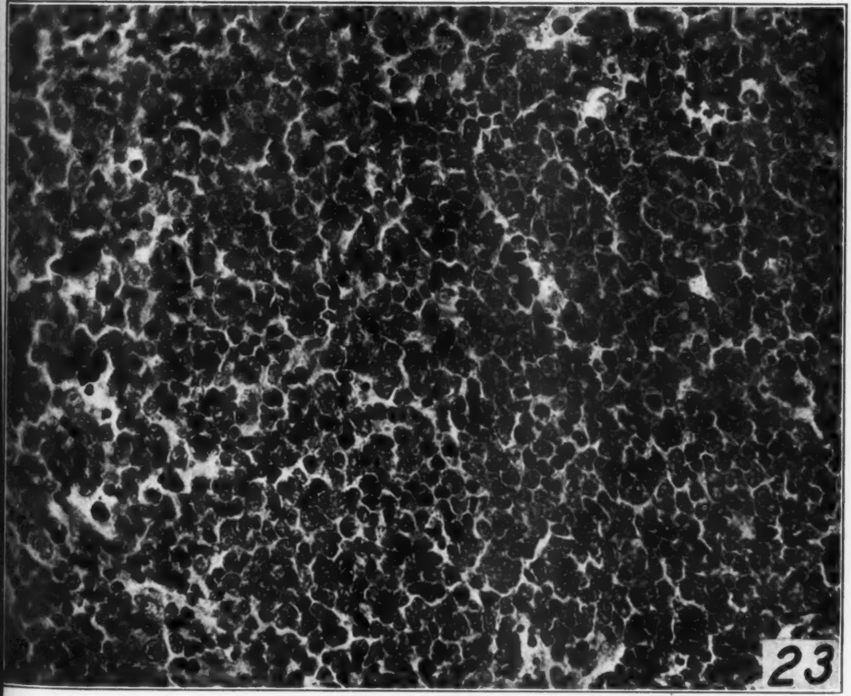
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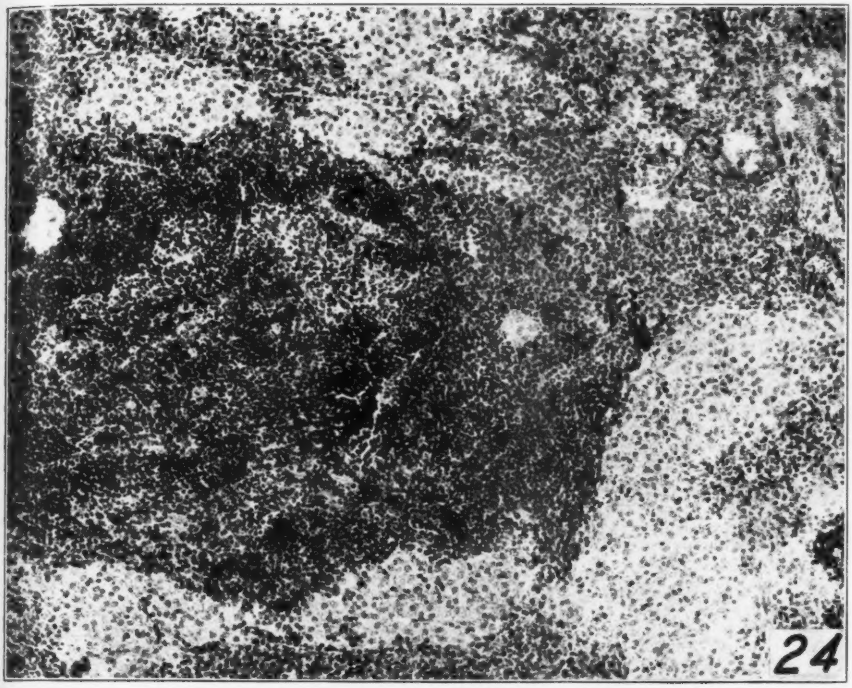
PLATE 141

FIG. 24. Germinal center and pulp of spleen 14 days after injection of the avian type of tubercle bacillus. Note the large germinal center, the cellular pulp and the monocytic tubercles in the germinal center and the pulp. Compare with Figs. 20 and 22. × 100.

FIG. 25. Higher power from edge of germinal center in Fig. 24. Mitotic figures present. Note admixture of small deeply staining, medium sized paler staining, and large pale staining, typically tuberculous monocytic cells. Compare with Figs. 21 and 23. × 400.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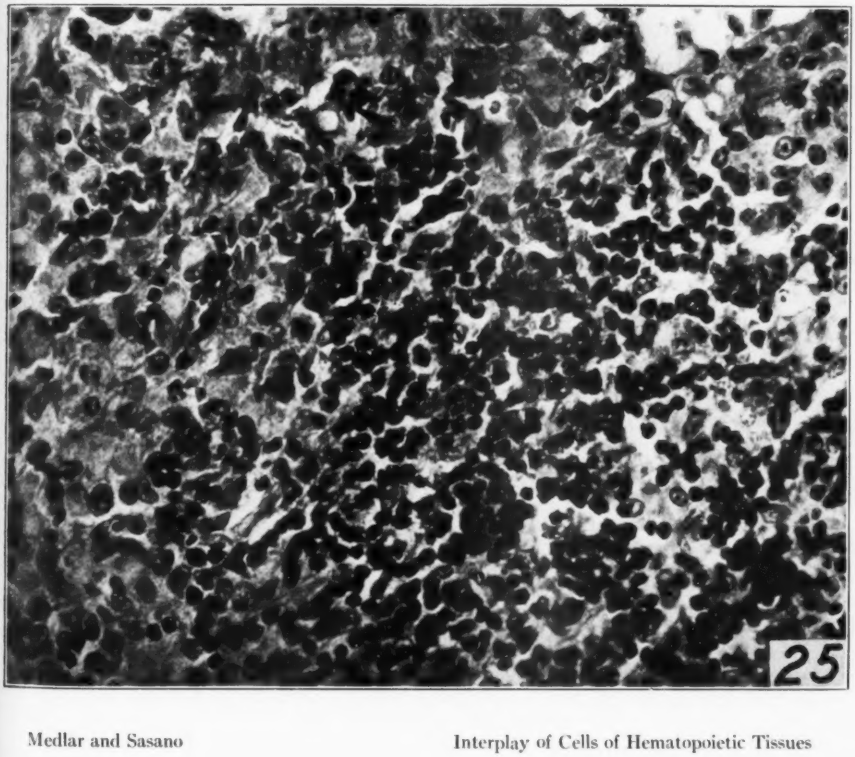
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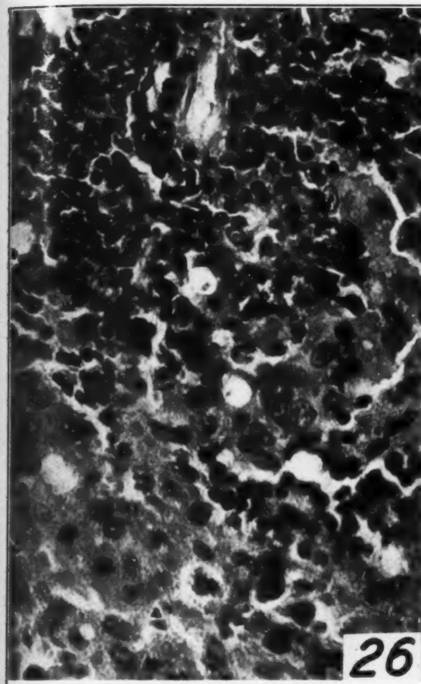
Plate 142

Fig. 26. Portion of central part of germinal center in Fig. 17, 3 weeks after inoculation of the avian type of tubercle bacillus. Note the artery, the small number of germinal center cells which remains, and the extensive encroachment of the large pale staining monocytes into the germinal center. Compare with Figs. 21 and 23. × 800.

Fig. 27. Sinusoid from spleen illustrated in Fig. 17. Note normal appearing endothelium at left and two mitotic figures lying free in the blood. × 800.

Fig. 28. Tubercle in pulp from spleen shown in Fig. 17. Note the accumulation of neutrophiles, especially in the central portion. With the death of the neutrophiles and the loss of staining power of their nuclear content the typical picture of caseation occurs. × 800.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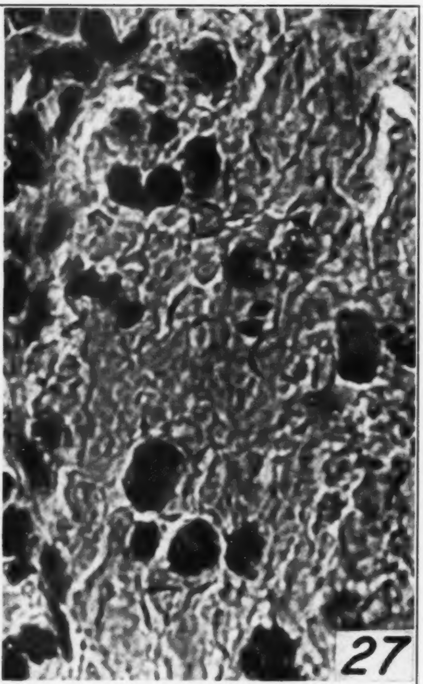
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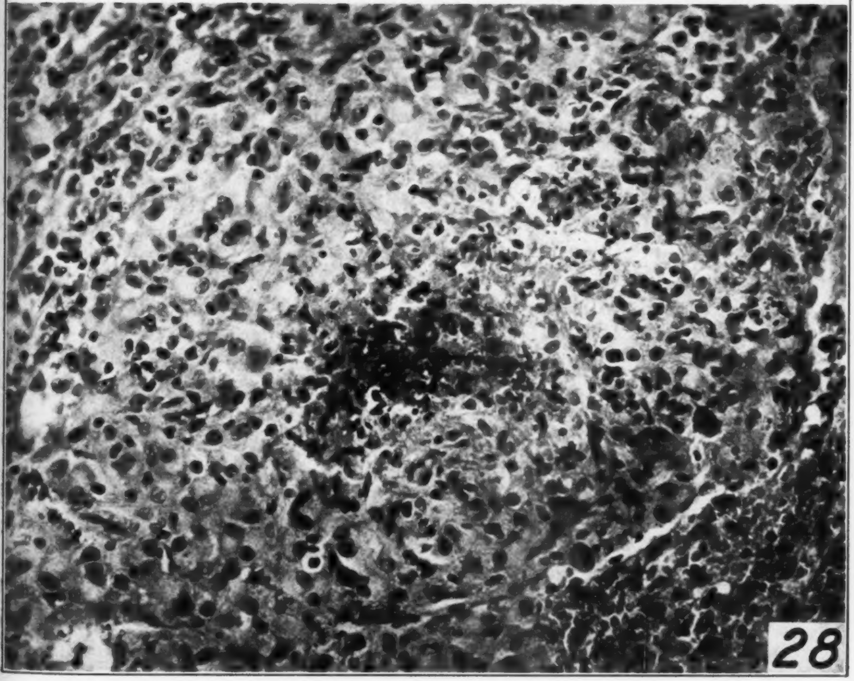
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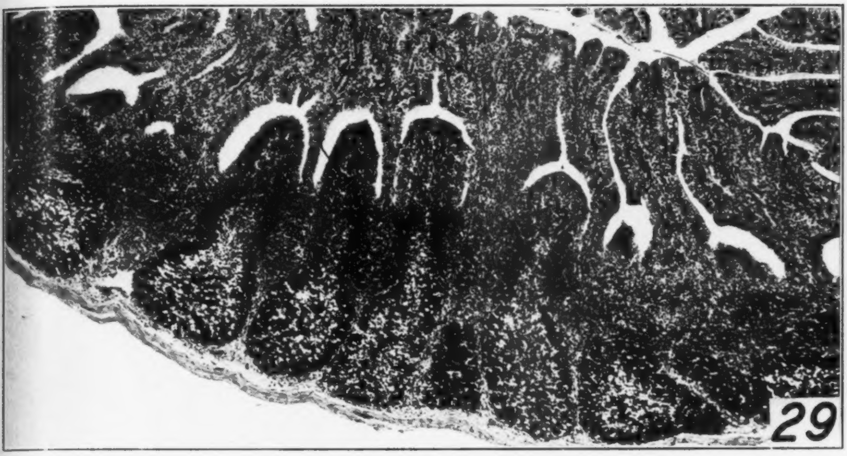
PLATE 143

FIG. 29. Appendix of normal rabbit. Note the flask shaped lymphoid apparatus with a serosal and mucosal portion. See description in text. × 50.

FIG. 30. Appendix of rabbit 2 weeks after inoculation of bovine type of tubercle bacillus. Compare with Fig. 29. Note great increase in size of serosal portion of lymphoid structure. × 50.

FIG. 31. Appendix of rabbit 3 months after inoculation of bovine type of tubercle bacillus. Note two tubercles in serosal portion and the small serosal and prominent mucosal areas in the lymphoid tissue not infected with tubercle bacilli. Compare with Figs. 29 and 30. × 50.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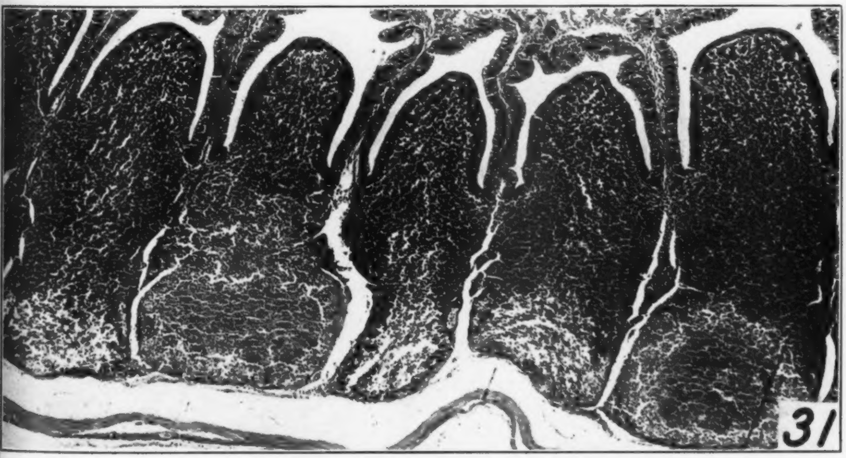
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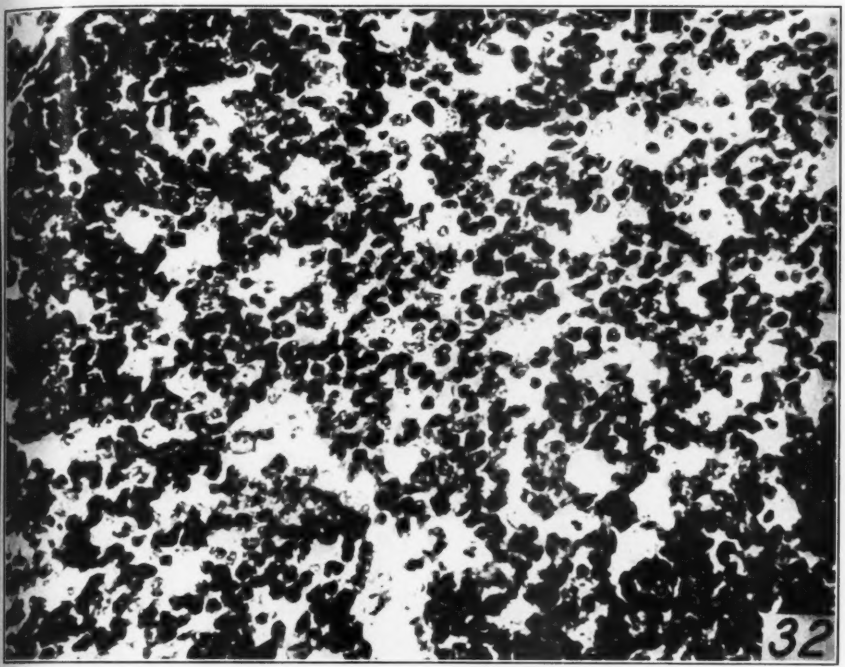
PLATE 144

FIG. 32. Serosal portion of lymphoid tissue from Fig. 29. × 500.

FIG. 33. Serosal portion of lymphoid tissue from Fig. 30. Note numerous mitotic figures. Compare with Fig. 32, especially the peripheral, deeply staining area. In Fig. 33 only a part of the peripheral area can be shown because of the marked hyperplasia. × 500.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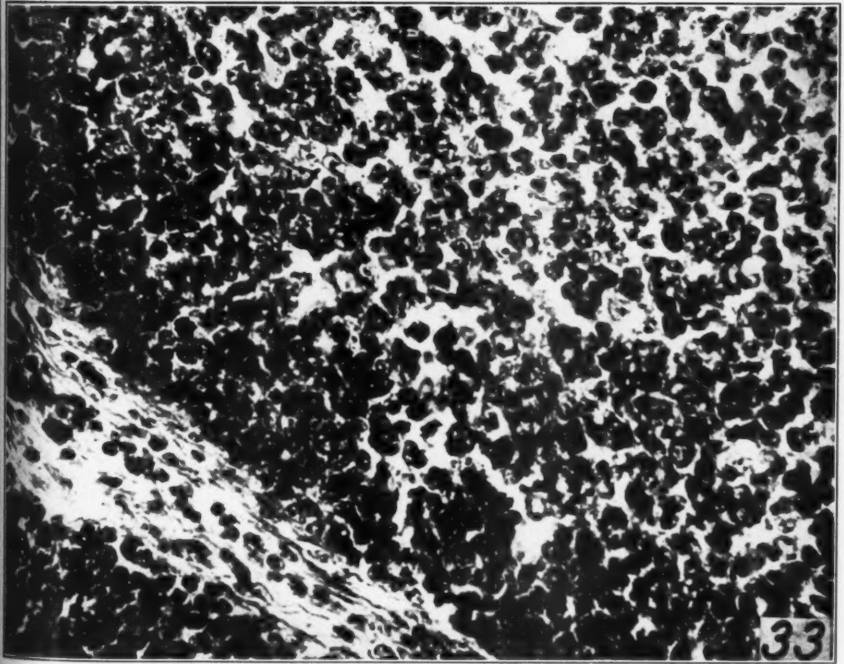
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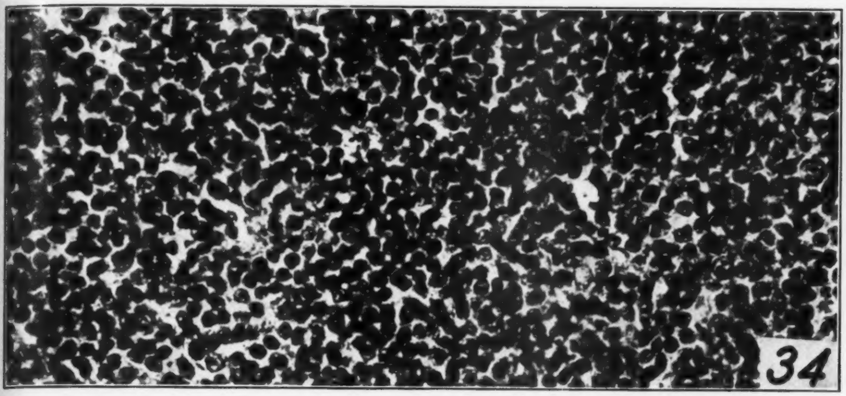
PLATE 145

FIG. 34. Mucosal portion of lymphoid tissue from Fig. 30. Note small uniform size of cells and absence of mitoses. This is more typical of lymphocytic type of tissue. Compare with Figs. 32 and 33. × 500.

FIG. 35. Monocytic tubercle from a different area of the appendix shown in Fig. 31. Note the well preserved character of the monocytes and their similarity to the same type of cell shown in Figs. 8, 9, 15 and 28. × 500.

FIG. 36. From the same appendix as Fig. 35. An area where the monocytes have largely necrosed and great infiltration of neutrophiles (irregular deeply staining nuclear masses) has occurred — a typical tuberculous abscess. With death of the neutrophiles and disintegration of their nuclei the typical picture of caseation appears as shown in Fig. 31. × 500.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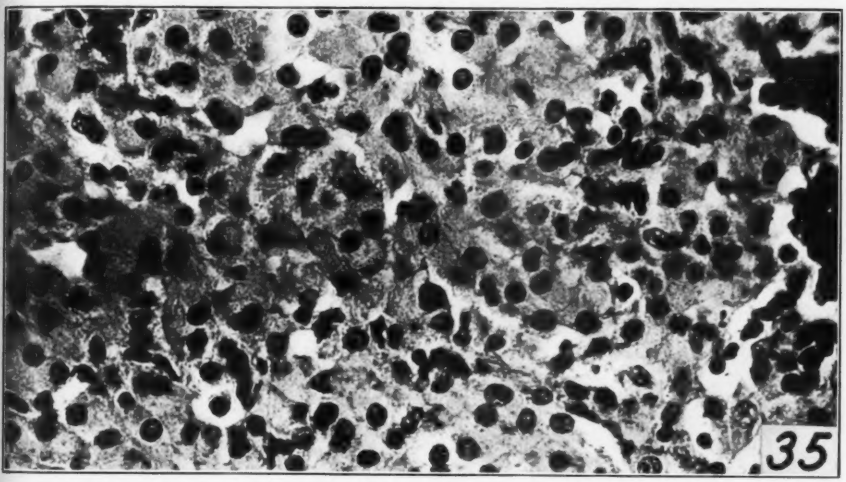
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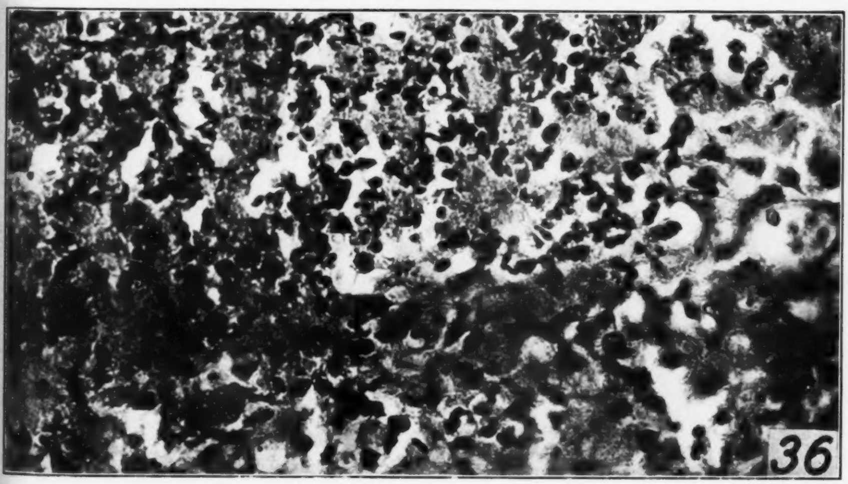
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LESIONS OF THE CARDIAC VALVES IN RHEUMATIC FEVER *

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The early descriptions of valvular disease dealt with the recognition of verrucae and the occurrence of gross valvular deformities. Aside from the absence of microscopic studies these descriptions were of limited value, first because of failure to recognize the importance of rheumatic fever as a cause of valvular disease and later because of confusion of valvular deformities due to rheumatic fever with those due to bacterial endocarditis, arteriosclerosis, syphilis, and so on. In the early part of the eighteenth century, Vieussens [1] gave clinical and pathological descriptions of mitral stenosis, aortic stenosis and aortic insufficiency. In the latter half of the century the thickening, whitening, loss of transparency and ossification of the semilunar valves were mentioned by various writers, including Morgagni,[2] de Senac,[3] and Baillie.[4]

Somewhat later Corvisart [5] described mitral stenosis and warty vegetations on various valves of an apparently rheumatic heart but believed the vegetations to be luetic. Laennec,[6] who termed these vegetations verrucae, and also Bertin,[7] failed to recognize their rheumatic origin but doubted the luetic theory of their formation. While Bouillaud [8] was not the first to observe the occurrence of heart disease in rheumatic fever, he most clearly recognized the association of various valvular lesions with that disease. He emphasized the thickening and stenosis of valves, the occurrence of verrucae at or near the free border rather than at the bases, and the occurrence of stages in valvular disease accompanied by organization and lime infiltration. However, there is considerable evidence that many of his cases were instances of bacterial endocarditis or of degenerative valvular disease. Watson [9] adequately depicted the most striking gross features of rheumatic valvular disease including the thickening, loss of transparency and pliancy, puckering, adhesions and vegetations.

During the latter half of the nineteenth century a number of reports dealt with the microscopic findings in rheumatic valvular disease. While the significance of bacteria in the causation of endo-

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carditis was understood, inadequate or imperfect bacteriological technique, incomplete clinical knowledge of the bacterial forms of endocarditis, uncertainty as to the bacterial etiology of rheumatic fever and the absence of rigid pathological criteria for the latter were some of the more important reasons for confusion. In the reports of Jaccoud,[10] Crocker,[11] Weichselbaum,[12] Klebs,[13] Birch-Hirshfeld,[14] and others, bacterial and rheumatic endocarditides were confused. The division of endocarditis into verrucous and ulcerative forms was not clear-cut because the former included bacterial as well as rheumatic lesions. Wyssokowitsch [15] and Orth [16] most clearly recognized that many of the cases of verrucous endocarditis showed no bacteria in the vegetations. The latter author stressed the importance of cellular proliferation in the valve and the avascularity of normal valves.

Detailed microscopic studies of valvular lesions in rheumatic endocarditis were reported by Achalme,[17] and by Königer,[18] and the formation of verrucae was discussed by Neumann,[19] and Ziegler.[20] The first mentioned reported bacterial infiltration of the valves and described what he considered to be the causative organism. Neumann conceived verrucae as being formed purely from the valve substance which had undergone fibrinoid degeneration. Ziegler, on the other hand, considered the verrucae to result from marantic thrombosis with deposition on the valve surface (thrombo-endocarditis). Königer's painstaking histological studies were based essentially on valvular disease in non-rheumatic infections. Only 2 definitely rheumatic cases were presented. The primary process was described as an endothelial necrosis. Verrucae were considered to be formed from the subendothelial tissues which proliferated, underwent coagulation necrosis and combined with thrombotic material deposited from the blood stream.

Later studies were characterized by a clearer delimitation of rheumatic endocarditis from subacute and acute bacterial endocarditis, from syphilitic disease and from arteriosclerotic valvular disease, especially of the Mönckeberg variety. This segregation resulted from the recognition of the Aschoff body as a specific criterion of rheumatic disease, from improved blood culture methods and more detailed clinical studies of bacterial endocarditis, from the discovery of the Wassermann reaction, and finally from more thorough knowledge of the histology of the normal valve. Nevertheless, there was still confusion between rheumatic and non-rheumatic cases in the

reports of Dewitzky [21] and of Felsenreich and von Wiesner.[22] The former, however, gave clear descriptions of the chronic valvular lesions in rheumatic fever and segregated them from those of Mönckeberg's ascending sclerosis of the aortic valve. The latter authors recognized some of the characteristic alterations of the elastica, and the lesions in the pockets of the valves and chordae tendineae attachments.

To Bulloch,[23] and especially to Carey Coombs,[24] we owe most of our recent knowledge of the histology of rheumatic valvular disease. Both of these authors, as well as Butterfield,[25] Thalhimer and Rothschild,[26] Gengenbach,[27] Clawson and Bell,[28] and others, described the presence of Aschoff bodies in the valves. Bulloch emphasized the occurrence of the earliest changes, not in the endothelial but in the subendothelial layers. These consisted of a coagulation necrosis with swelling and homogeneous transformation of the ground substance. Coombs drew attention to the proliferative and exudative reactions in the valves as well as in other regions of the heart. The proliferative reaction was the more marked. The deeper structures reacted before there was evidence of injury to the endothelial surface. This observation, among others, led to the concept of a deep valvulitis first and verrucous formation later. Following Poynton and Paine,[29] Coombs believed that infection came by way of the coronary branches and not superficially from the circulating blood. Like Poynton and Paine he also described what he believed were bacteria in the valve.

Similar descriptions were given in the more recent studies of Clawson, Bell and Hartzell,[30] and by Klinge [31] and his coworkers. The former authors described acute and healing lesions in rheumatic endocarditis and old valvular defects. Klinge described an acute stage of valvular inflammation with subendothelial focal and banded swellings, a later granulomatous stage with Aschoff bodies or diffuse cellular infiltrations, and finally a stage of scarring. The valvular lesion was considered primary and the verrucae secondary.

The detailed description of valvular lesions given by Ribbert [32] is essentially like that of Königer. Benedict [33] gave a differential pathological description between rheumatic and syphilitic disease of the aortic valve. He particularly emphasized that in lues there was an increase of endocardial elastica, whereas in rheumatic fever the elastica was torn and diminished in amount. Leary [34] drew attention

to palisade cell formations along the contact edges of the valves which he believed represented a specific early rheumatic reaction. Of the 3 cases which he reported, only 1 was definitely rheumatic, and that patient died of an acute infection.

Many of the more recent studies have concerned themselves with investigating grossly normal valves from patients dying of acute infections, or the grossly normal portions of valves which elsewhere showed macroscopic abnormality. Such reports were made by Baldassari,[35] Holsti,[36] Böhmig and Krückeberg,[37] de Vecchi,[38] and by Waldow.[39]

In summarizing and appraising the above mentioned reports on the pathogenesis of rheumatic valvulitis, they may be divided into two periods. During the period preceding the discovery of the Aschoff body as the specific lesion in rheumatic fever, the reports were largely confused by the failure in many cases to differentiate the valvular lesions of rheumatic fever from those occurring in other endocarditides as well as from other degenerative valvular changes. Following the discovery of the Aschoff body confusion still persisted: (1) because this lesion is not invariably present in rheumatic fever, especially during the less active stages; (2) because the unfortunate classification of endocarditis into verrucous and vegetative led to no sharp differentiation of the several types; (3) accurate descriptions of the normal structure of valves were not available, with the result that the pathological changes were seldom referred to with precision in respect to the layers of the valve involved; and (4) age period changes occurring in normal valves received practically no attention, thus adding considerably to the difficulty of discerning the lesions due essentially to the chronic rheumatic process.

In a series of studies reported by one of us (L. G.) with collaborators, attention was drawn to the pathogenesis of various rheumatic lesions occurring in the heart, *viz.*, of the myocardium [40] (Aschoff bodies), blood vessels,[41] large vessel roots,[42] auricles,[43] conduction system,[44] pericardium [45] and valve rings.[46] In these investigations a description of the normal histology and topography as well as of the age period changes in these sites was included. In following the life cycle of these lesions in indisputable cases of rheumatic fever certain stigmata of active as well as healed rheumatic lesions were demonstrated. These stigmata of healed rheumatic lesions are particularly

important in establishing the essential rheumatic nature of a given valvular lesion. On the basis of such studies it now becomes possible sharply to define rheumatic from non-rheumatic hearts, even in the absence of Aschoff bodies or a typical clinical history.

According to the definitions of Gross and Kugel,[47] the auriculoventricular valve leaflet consists of the fibro-elastic structure immediately distal to the auricular myocardial wedge, and the semilunar valve leaflet consists of the fibro-elastic structure attached to the subjacent ventricular myocardium through the intermediary of annulus interdigitations (Figs. 1 and 2). This definition includes the valve ring as the proximal portion of the leaflet.* The ring lesions in rheumatic fever recently described by the present authors, therefore, can be legitimately considered as part of the valvular lesion as a whole. These rheumatic ring lesions have been described separately for purposes of clarity, but reference should be made to the complete report in order to preserve a logical continuity with the descriptions to be given of the lesions in the remainder of the leaflet.

Briefly to recapitulate the findings in non-rheumatic and rheumatic rings, the following points should be borne in mind: Normal valve rings are practically devoid of inflammatory cells. The ring spongiosa in the semilunar cusps almost invariably consists of a gelatinous tissue and is generally sharply separated from the adjacent fibrous structure (annulus). In the auriculoventricular valves the spongiosa component is generally inconspicuous. Blood vessels with muscular walls are never seen in the normal rings. The incidence of capillaries in these rings, as determined in 100 normal hearts, is as follows: anterior mitral valve ring, 1 per cent; posterior mitral valve ring, 2 per cent; aortic valve ring, 0 per cent; tricuspid valve ring, 14 per cent; and pulmonary valve ring, 7 per cent. When present, the capillaries are generally few in number, small and circular on cross-section, and in structure easily differentiated from granulation tissue capillaries.

On the other hand, the characteristic features of the ring lesions in rheumatic fever are briefly as follows: The rings are almost invariably infiltrated with inflammatory cells, capillaries and blood vessels. The latter are sometimes of a characteristic type. The inflammatory process generally spreads into the contiguous valve leaflets

* A full description and delimitation of the valve rings were reported by Gross and Kugel.[47]

as well as along the annulus extensions of the aortic root. These contiguity extensions of the inflammatory process are present in the septum fibrosum as well as in the intervalvular fibrosa (the collagenous link between the aortic and mitral valves). The subvalvular angles show characteristic lesions, termed reduplications. These are frequently inflamed and vascularized. Scarring of the ring occurs, with obliteration of the ring spongiosa. The extent of these inflammatory phenomena is determined by the clinical course of the disease.

Bearing in mind, therefore, this intimate relation of the ring lesions to those that occur in the remainder of the valve leaflets in rheumatic fever, we are now in a position to take up the description of the gross and microscopic changes that take place in the latter and to discuss their significance with regard to the pathogenesis of rheumatic valvulitis. The description of these lesions will be preceded by a discussion of the gross and microscopic findings in normal valves, together with a consideration of their age period changes.

Material and Methods

The material consisted of 40 non-rheumatic control hearts and 97 rheumatic hearts. Seventy-one of the latter were from active rheumatic cases and showed Aschoff bodies in the myocardium, and 26 showed chronic valvular disease of the typical rheumatic variety but without evidence of activity either clinically or pathologically and with no demonstrable Aschoff bodies in the myocardium. The grouping as to activity and inactivity was based on the criteria outlined by Rothschild, Kugel and Gross.[48] Particular care was taken to avoid material that in any way indicated the possibility of a coexisting bacterial endocarditis or syphilis. A careful study of the clinical records and pathological specimens made it possible to divide the rheumatic cases into the following groups:

Group I. Active cases where death took place during the first attack (12 cases).

Group II. Active cases where one preceding attack occurred within 1 year of the fatal outcome (7 cases).

Group III. Active cases where one previous attack occurred at least 2 years previous to the fatal outcome (11 cases).

GROUP IV. Active cases with a history of repeated attacks, death occurring during an acute recurrence (13 cases).

GROUP V. Active cases where death was caused by decompensation without clinical evidence of a final acute attack. In some of these cases there was no previous history of rheumatic fever (28 cases).

GROUP VI. Inactive cases of chronic valvular disease of the typical rheumatic variety (26 cases).

The sections from which these studies were made were cut according to the standardized technique of Gross, Antopol and Sacks,[49] and the technical procedures were those previously described by Gross and Ehrlich.[40]

AGE PERIOD CHANGES IN THE GROSS APPEARANCE AND HISTOLOGICAL STRUCTURE OF NORMAL VALVES

A study of the gross appearance of normal valves revealed only such alterations as could be ascribed to increasing age and tension. In about half of the cases, particularly in the older age periods, the uniform slenderness and transparency of the valve cusps were slightly altered by the occurrence of isolated patches of whitish opaque thickening. These thickenings were generally not notable. They were situated at various portions of the valve leaflets but most frequently at the closure line and free margin. When present at the free margin, the thickening obscured the normal concave scalloping and rendered the margin either straight or convex.

Not infrequently there was broadening of the heads of the chordae tendineae that were attached to the region of valvular thickening. In addition to these, there were occasional yellowish lipoid flecks which spotted the valve ring, both aspects of the valve cusps, and especially the valve pockets.

The pocket of the normal valve generally formed a sharp narrow angle which was traversed only occasionally by an isolated bridge of fibrous tissue. Except for the lipoid flecks just mentioned, there were none of the irregularities that will be described as occurring in the pockets of the various rheumatic valves.

The histological structure and topographical relations of normal human heart valves have been described in detail by Gross and

Kugel.[47] Briefly considered, they are characterized by the following features: All valve leaflets carry as their main backbone a dense collagenous layer called the fibrosa (Figs. 1 and 2). Adjacent to the fibrosa layer and sometimes not clearly distinguished from it, there is a zone of loose connective tissue called the spongiosa layer. This is situated on the auricular aspect of the auriculoventricular valve fibrosa as well as on the ventricular aspect of the semilunar valve fibrosa. In the semilunar cusps the spongiosa layer may be so conspicuous as to constitute a sharply defined zone of loose gelatinous tissue. In the auriculoventricular cusps the spongiosa layer is frequently quite inconspicuous in its proximal two-thirds and becomes discernible and widened generally only in the presence of inflammation. The distal third, or tip of the valve, generally consists of a gelatinous expansion of the spongiosa layer. On the auricular surface of the auriculoventricular leaflets there lies a fibro-elastic mantle of various thickness, called the auricularis layer. This is covered by a flat layer of endothelial cells. The ventricular surface of these leaflets is covered by a much thinner layer of fibro-elastic tissue called the ventricularis. This, in turn, is also covered by endothelium. The ventricular aspect of the semilunar cusps is clothed by a fibro-elastic mantle somewhat more delicate than the auricularis of the auriculoventricular cusps. This is called the ventricularis of the semilunar cusps. The arterial aspect of the semilunar cusps is covered by an even more delicate elastic mantle. Inasmuch as the auricularis layer of the auriculoventricular valves and the ventricularis layer of the semilunar valves are the first to be impinged by the blood stream, these will be referred to as the "proximal layers." For similar reasons, the ventricularis layer of the auriculoventricular valves and the arterialis layer of the semilunar valves will be referred to as the "distal layers." These terms are not to be confused with the proximal and distal portions of the valves, *i.e.*, the insertions and tips, respectively.

Normal valve leaflets are poor in cells. This is particularly noticeable in the fibrosa layer. In spite of the controversial reports on the existence of blood vessels in valves, the available evidence leaves little room for doubt that, apart from the sparse capillaries mentioned above as occasionally occurring in the valve ring, blood vessels are seldom, if ever, present in normal human heart valves.[50] It may be stated in passing that the high incidence of valve vascularization,

as recently reported by Wearn *et al.*,[51] can be adequately accounted for chiefly by the inclusion into the statistics of vessels supplying the auricular myocardial wedges of the auriculoventricular valves, the occasional presence of ring capillaries, and acceptance of extinct or mild valvulitides as normal material.

With advancing age periods, the various layers of the valves become progressively poorer in cells and take on the following changes: The strata become increasingly well defined; the semilunar cusp spongiosa becomes more and more fibrous and elastified; the auriculoventricular valve ring spongiosa and the spongiosa situated opposite the chordae tendineae insertions become loose and often the seat of fat deposits; the elastic membranes become heavier and longer; the auricularis and often the ventricularis layers become appreciably denser, more collagenous and thickened; and the collagenous fibrosa undergoes degenerative lipoid changes. The point last mentioned is inevitably associated with the calcium salt deposition of the later age periods.

The tips (distal portions) of the valve leaflets become somewhat thickened with advancing age periods. In the auriculoventricular valves, particularly the mitral, this thickening is due to two processes, *viz.*, fibro-elastification of the auricularis layer at the closure line, and absorption of thickened chordae tendineae insertions into the fibrosa. The fibro-elastified thickening at the site of the closure line is generally quite dense, somewhat oval on cross-section and never contains inflammatory cells or blood vessels. The chordae tendineae insertions beneath the tips of the leaflets show reduplications of their endocardial covering. These may become so exaggerated and agglutinated to one another as to thicken appreciably the tip of the cusp. As will be shown subsequently, however, thickening at the tips of rheumatic valves is due to an entirely different process.

Other age period changes in the auriculoventricular cusps are the formation of delicate crescentic reduplications of the ventricularis layer around the insertions of the chordae tendineae of the second and third order. In the semilunar cusps advancing age produces a more gradual and more delicate elastification of the ventricularis layer, particularly near the semilunar folds. The noduli Arantii and Morgagni become markedly elastified and hyalinized. Here again inflammatory phenomena are absent.

Gross Appearance of Rheumatic Valves in Group I

(12 Active Cases Where Death Took Place During the First Attack)

The most frequent gross alteration of the valve leaflets observed in this group was a definite diffuse thickening. This was invariably present in the mitral and aortic valves and in eight of the twelve tricuspid valves, but the pulmonic valves, with one exception, appeared normal. In general, the thickening was uniform throughout the cusp, but in the mitral valve this change was accentuated at the closure line by the formation of a fine ridge.

The normally concave, scalloped, sharp margins of the auriculoventricular valves were almost invariably thickened and straight. In about one-third of the mitral valves the peripheral portion of the leaflet became slightly protuberant to form an overhanging shelf.* In a few of the tricuspid valves the scalloped concavity of the free margin was likewise obliterated, but in no instance was there shelf formation.

The auricular surface of the auriculoventricular valves occasionally showed an irregular corrugation. In 3 cases moderate gross vascularization was noted on the superficial aspects of the auricular surface of the mitral valve and once on the tricuspid valve.

In the aortic valve the sharp margin of the cusps frequently became thickened and rounded, and in about one-third of the cases this rolled margin was slightly inverted toward the sinus pocket. In 2 cases the semilunar folds were elevated toward the free margin, and once the free margin showed a distinct notch at about the site of the nodulus.

Verrucae were present on all of the mitral valves, on eight of the aortic and on seven of the tricuspid. The verrucae were fine, pinhead-sized, yellowish and gray elevations which usually fused with each other. Many of them were fresh; some showed evidences of healing. The verrucae were situated at the closure line, free margin, or at both sites. Extension of the verrucae from the mitral leaflets to the insertions of the chordae tendineae was frequent. They occasionally extended around the free margin to the ventricular aspect of the

* The term "shelf" is employed to indicate the projection of valve tissue over the first order chordae tendineae insertions in such a manner as to overhang the latter. In contradistinction to the "shelf," prominence of the closure line is referred to as a "ridge."

valve. In 1 case there was a fresh verrucous deposit in the pocket of the posterior mitral cusp. The verrucae tended to form conglomerate mounds on the noduli Arantii of the aortic valve and from there extended in rows along the semilunar folds. Frequently the verrucae occurred in isolated fashion, affecting only a single cusp of a valve or even only part of a cusp. In one instance the verrucae on the tricuspid valve extended onto a papillary muscle.

The pockets of the valves in this group were generally normal. In 1 case, as has been mentioned, there were fresh verrucae in the posterior mitral pocket. In 2 cases there were a few irregular folds and ridges in the sinus pocket of the aortic valves.

In about one-third of the cases in this group there were distinct abnormalities of the chordae tendineae. The occurrence of verrucae at their attachments to the valve has already been mentioned. In 1 case there were organizing verrucae extending halfway down the chordae. Not infrequently the chordae tendineae were distinctly thickened at their attachments to the valves (ham shaped), particularly where these attachments were close to confluent verrucae on the valve. Sometimes they were shortened and rarely was there fusion of isolated chordae. In 1 case the chordae tendineae of the septal leaflet of the tricuspid valve were agglutinated to the underlying endocardium (Fig. 3).

Microscopic Appearance of Rheumatic Valves in Group I

The thickening of the valve leaflets in this group was due to inflammation, edema and hypercapillarization of the proximal layers of the valve (auricularis layer of the auriculoventricular valves and the ventricularis layer of the semilunar valves) together with similar involvement of the spongiosa layer (Figs. 4 and 5). The lesions obviously represented a contiguity process from the ring and generally extended along the entire length of the leaflets. The vascularization of these layers, as well as the others to be described, consisted almost entirely of capillaries. Occasionally vessels with muscular walls were noted. These were sometimes of the intimal musculo-elastic hyperplastic type. The inflammatory cells were chiefly lymphocytes. In some cases, however, polymorphonuclear leukocytes predominated. Plasma cells, fibroblasts, macrophages and other mononuclear cells were occasionally seen.

Besides edema, hypercapillarization and exudate, the spongiosa

layer sometimes showed elastica condensation and disruption. Eosinophilic swelling of the collagen was not infrequently seen. This generally involved the spongiosa layer in its main body as well as at the tip of the leaflets. The tip was rarely scarred.

A prominent feature of this group was inflammatory involvement of the fibrosa layer (Figs. 4 and 5). This was greatest in the aortic valve and occurred chiefly in the zone that is adjacent to the spongiosa. It consisted of capillarization, inflammatory cell involvement and sometimes elastic tissue formation. In a number of cases the fibrosa layer contained large swollen cells with basophilic cytoplasm between the collagen bundles. These cells not infrequently bore a resemblance to those that form the Aschoff body. Indeed, in a number of instances, typical Aschoff bodies were seen in the fibrosa layer. This occurred most frequently in the tricuspid and pulmonic valves.

In most of the auriculoventricular valves the ventricularis layer was thickened, inflamed and vascularized. The arterialis layer of the semilunar cusps was also frequently thickened and somewhat inflamed. Vascularization of this layer, however, was infrequent.

The tips or distal portions of the valves in this group generally retained some of their normal spongy structure, even though many of them were the seat of inflammatory lesions including Aschoff bodies. By contrast it will be shown that in the groups to be described, the mitral, aortic and tricuspid valve tips showed increased fibrosis.

In discussing the incidence of verrucous lesions in all the clinical groups studied, as determined by microscopic examination, reference will be made only to those that were fresh, *i.e.*, still possessed eosinophilic hyaline material with or without evidence of organization. The sections cut were generally selected with a view toward including such lesions. Furthermore, as is to be expected, verrucous lesions were sometimes noted microscopically when they were overlooked on gross examination. As a consequence, there will be a discrepancy in the incidence of these lesions as listed under the gross and histological findings, respectively. The latter undoubtedly present a more accurate picture of the actual incidence of those lesions that still contained unorganized verrucous material.

Histological studies on the nature of these verrucous lesions suggest that neither platelets nor fibrin are concerned in their formation. They appear to be due to a disintegration and fusion of proliferating

cells on the superficial layers of the valve leaflets, generally at their most exposed portions, or at sites that form a cul-de-sac in which blood eddies or stasis may occur. Together with this fusion of proliferated cells (endothelium, fibroblasts and other cellular constituents), swelling and eosinophilic changes take place. Whether or not constituents from the plasma are deposited within this material, it is as yet impossible to determine. It appears that the verrucous material is extruded from the valve leaflet because of its swelling and because of cicatrization and contraction of the underlying tissues. Another contributing factor leading to the extrusion of the verrucous material may be the accumulation of inflammatory exudate and the proliferation of swollen basophilic endothelial cells at the base of the verrucae. The fresh verrucae are seldom covered by endothelial cells. The healing stages consist of fibroblastic invasion of the verrucous material with, eventually, complete replacement by scar tissue. Typical granulation tissue capillaries may invade the verrucae (Fig. 6).

The verrucae as a whole in this group were quite extensive and fresh. Moreover, their incidence was high. In 9 of the 12 cases these lesions were present at the closure line of the anterior mitral leaflet. In 3 of the 9 cases they were extensive and spread completely around the tip of the cusp on the ventricularis surface. In 3 additional cases verrucae were observed on the chordae tendineae attachments to the leaflets. Thus, verrucae were observed in every case of this group on the anterior mitral cusp or its chordae tendineae insertions. In 9 cases there were verrucae on the posterior mitral leaflet. Some of these were at the extreme tip of the cusp. Most of them, however, were on the closure line. One additional case showed verrucae on the chordae tendineae insertions (Fig. 5), and another in the posterior mitral pocket. In 9 cases verrucae were present on the closure line of the aortic cusps, and an additional case showed verrucae in the aortic pocket. In 4 cases verrucae were present on the tricuspid valve. An additional 4 cases, however, showed verrucae in the tricuspid pocket. In 2 cases these were seen on the chordae tendineae insertions. Including the pocket and the chordae tendineae insertions, verrucae were present on some part of the tricuspid leaflet in 8 of the 12 cases in this group. Only one pulmonic valve showed verrucae on the closure line. In 2 additional cases, however, verrucae were present in the pulmonic pocket. It is of considerable interest to

note that when the inflammatory lesion did not extend beyond the ring or the base of the pulmonic valve, verrucae tended to occur either in the pocket or in the subvalvular angle. Thus, there appeared to be a tendency for verrucae to localize at a level corresponding to the distal extension of the inflammatory process within the leaflet. This point will be discussed more fully.

As mentioned above, verrucae were noted once in the aortic pocket, once in the posterior mitral, four times in the tricuspid and twice in the pulmonic. Apart from these lesions, most of the valve pockets in this group showed endocardial reduplications, often with mild inflammatory cell infiltrations. These were most notable in the semilunar pockets. At times, the reduplication showed eosinophilic degeneration. This occasionally involved the elastic limiting lamellae, chiefly in the semilunar pockets. Another even more characteristic pocket lesion is polypoid formation (Fig. 8). On cross-section this consists generally of inflamed, finger-like processes, giving the impression of minute polypi. Whether, indeed, these are polypi or whether the spaces between the finger-like processes represent merely dipping down of the endocardium to form vascular channels, it is difficult to determine. The early stages in their formation apparently consist of the extrusion of tiny endocardial hillocks into the valve pocket, *i.e.*, toward the cardiac lumen. These hillocks become elongated and their bases may undergo eosinophilic swelling and fusion. These polypoid lesions were noted in the aortic pocket four times, in the tricuspid once, and in the pulmonic three times.

The incidence of verrucae on the chordae tendineae insertions has already been mentioned. In addition, the chordae tendineae of the anterior mitral cusp showed inflamed reduplications in half the cases. Three of these were vascularized. Four of the cases showed vascularized inflamed reduplications around the insertions of the chordae tendineae in the posterior mitral cusp (Fig. 6). Only 1 case showed reduplications on the tricuspid valve chordae tendineae. Not infrequently the chordae tendineae insertions were agglutinated to one another through the intermediary of verrucous material. Cross-sections of these chordae tendineae often showed swollen basophilic cells scattered between the collagenous bundles. These cells were similar to those described in the inflamed fibrosa layer of the valve.

Although gross vascularization of the valves was inconspicuous in

this group, its incidence microscopically was extraordinarily high. Thus, in the single sections which generally represented each cusp studied, 11 cases of this group of 12 showed blood vessels (generally capillaries) in the anterior mitral cusp, 12 in the posterior mitral, 9 in the aortic, 8 in the tricuspid and 6 in the pulmonic. Moreover, in almost every section blood vessels were present in the valve ring. Thus, if the ring is considered, as it should be, the proximal portion of each cusp, it may be said that almost invariably every cusp of the heart showed blood vessels in this group.

Considered as a whole, the mitral and aortic valves generally showed the widest involvement. However, the leaflets of the tricuspid valve were quite frequently more intensely inflamed than were those of the other valves. In this respect, there was a similarity to the very flagrant involvement of the tricuspid ring. Furthermore, the inflammation seemed to be most severe toward the root of the valve, and edematous widening and hypercapillarization of the auricularis layer were occasionally present. The pulmonic valve generally showed milder lesions. Both the exudative phenomena as well as capillarization were subdued. The most extensive lesions were found in the spongiosa layer.

Summarizing the conspicuous features of the valvular lesions as a whole in Group I, the following points should be noted: The ring lesions * were extensive, consisting of pronounced capillarization and infiltration with inflammatory cells, sometimes with edema. Blood vessels of the muscular type were infrequent. Aschoff bodies were present in about 10 per cent of the rings. There was little scarring. Practically all the rings and subaortic angles showed lesions. In the latter site, reduplications, when present, were generally not multiple. Approximately half the cases showed involvement of the intervalvular fibrosa.

The lesions in the remainder of the valves generally consisted of intense inflammation, edema and hypercapillarization which involved all portions of the leaflets about equally. There was considerable involvement of the spongiosa and fibrosa layers. Aschoff bodies and eosinophilic swelling of collagen were present in these layers in some cases. The tips of the valves were seldom scarred. The incidence of verrucae was high. These lesions were extensive, fresh, and

* The descriptions of the ring lesions given in the summary of each group are abstracted from the detailed report by Gross and Friedberg.[16]

showed little organization. Lesions in the pocket and chordae tendineae were frequent. Capillarization of valve leaflets was almost universal.

Gross Appearance of Rheumatic Valves in Group II

(7 *Active Cases Where One Preceding Attack Occurred Within 1 Year of the Fatal Outcome*)

The most constant gross alteration in this group was a thickening of the valve cusps. Compared with Group I, the thickening was somewhat greater. In no case was the mitral, aortic or tricuspid valve of normal slenderness and translucency. The pulmonic valves, which in Group I were generally normal, revealed in the cases of this group a juicy, succulent consistence with occasional thickening and opacity. Gross vascularization was observed in every case and occurred chiefly in the mitral valve.

Ridge formation at the closure line of the mitral valve, as well as the presence of an overhanging shelf, was quite frequent and much more advanced than in Group I. Corrugation of the auricular surface occurred to the same degree as in the first group.

The aortic valve showed alterations similar to those in the first group, but these were much farther advanced. The thickening was greater and verrucae were present in all cases. The semilunar folds of the aortic cusps were invariably elevated toward the free margins or were completely obliterated (Fig. 10). Rolling and inversion of the free margin and notching at its center were present in 4 of the 7 cases. The notching was due to inversion of the nodulus Arantii into the sinus pocket. Occasionally the nodulus was greatly hypertrophied, forming a knob near the middle of the free border. In 2 cases there was adhesion of the commissures.

Fresh, healing or healed verrucae were invariably present in this group. The location of the verrucae was the same as in Group I except that they were often superimposed upon the ridge on the mitral valve. Furthermore, a double row of verrucae was present on a few valves, one representing healed and one fresh lesions. In addition to the thickening of its cusps, the tricuspid valve invariably revealed verrucae, isolated or in a row.

Abnormalities in the pockets of one or more valves were present in every case. In 4 of the 7 cases there were fresh or healing verrucae in one or more of the valve pockets. These appeared either as tiny,

pinhead-sized yellowish deposits, or as small, yellowish smooth mounds. Occasionally there were other irregularities forming tiny nodular ridges or folds which distorted the normal sharpness of the pocket angle. The alterations of the chordae tendineae were similar to those in the previous group, but the changes appeared more frequently and were more advanced.

Microscopic Appearance of Rheumatic Valves in Group II

As noted above, the cusps in this group were somewhat more thickened than in Group I. The thickening was due to the following factors: The proximal layers of the valves generally showed one or more reduplications (Figs. 7, 8 and 9). These were frequently fibroelastic and occasionally fused with the underlying, generally widened spongiosa. These layers contained numerous inflammatory cells as well as muscular blood vessels, many of which showed typical intimal musculo-elastic hyperplastic changes.* Indeed, the high incidence of the latter is one of the most conspicuous features of this group. The inflammatory involvement of the valve leaflets showed definite contiguity with the increased ring lesions present in this group. The spongiosa participated notably in these changes. In all the cusps this layer was generally considerably widened with inflammatory exudate and profusely vascularized. The exudative phenomena were even more prominent in this group than in the previous one. As in the latter, the inflammatory cells were chiefly lymphocytes. Occasionally the polymorphonuclear leukocytes predominated. Fibroblasts, plasma cells and macrophages also occurred.

In a previous report it was shown that one of the characteristic features of the ring lesions in Group II is the formation of multiple vascularized elastified reduplications at the subvalvular angles (Figs. 8 and 9). A somewhat similar process takes place in the auricularis layer over the ring region of the auriculoventricular valves (Fig. 7). Not infrequently a prolongation of these multiple vascularized elastified reduplications produces considerable thickening of the cusps.

Another notable feature in this group was the definite involvement of the fibrosa layer. This was generally vascularized or capillarized along the zone that is contiguous with the spongiosa layer.

* For a detailed description of these vascular lesions see Gross, Kugel and Epstein.[41]

The fibrosa sometimes showed edema, elastica condensation and disruption. Inflammatory cells were numerous. Not infrequently one could trace the vascularization and inflammation of the fibrosa layer along the intervalvular fibrosa. This in turn showed a contiguity process from the aortic ring. In two instances the fibrosa lesion in the mitral leaflet showed such distinct whorling and inflammatory infiltration as to resemble a syphilitic contiguity process from the root of the aorta.

Aschoff bodies were present either in the fibrosa or spongiosa layer in several of the cases. These occurred with approximately the same frequency in all four valves. In addition, the annulus, particularly of the semilunar cusps, occasionally showed eosinophilic swelling of the collagen. Intercryptic cells resembling those seen in Aschoff bodies were also noted in the fibrosa layer, chiefly of the semilunar cusps.

Delicate reduplications of the distal layers of the valves (ventricularis layer of the auriculoventricular cusps and arterialis layer of the semilunar cusps) were frequently present. These reduplications were often inflamed and, in about one-third of the cases, showed vascularization.

A distinct difference from the previous group is the fact that the tips of the cusps almost invariably showed considerable fibrosis and some elastification. Thus, the normally gelatinous tip, which can be considered as forming an extension of the spongiosa layer, was almost always converted into a dense, fibrotic or fibro-elastic, often vascularized, inflamed structure. When the tip becomes collagenous (and, to a lesser degree, elastified), it fuses with a similarly altered auricularis layer to form a thickened ridge. Elastic lamellae from the auricularis and spongiosa layers, together with smooth muscle bundles, may be seen curving beneath this thickened ridge. The tips of the pulmonic cusps, however, rarely showed this collagenous transformation.

The incidence of verrucae still possessing a hyaline structure was highest in this group. Furthermore, the verrucae were generally quite broad and often extended from the closure line around the tip of the leaflet to the ventricularis surface on the auriculoventricular cusps. Verrucae were present on the anterior mitral leaflet in 6 cases. In 2 cases verrucae were noted on the chordae tendineae attachments to this leaflet. Every case showed verrucae on the pos-

terior mitral cusp, two in the posterior mitral pocket, and two on the chordae tendineae insertions. Verrucae were present on all the aortic valves. These lesions were also generally broad and extended around the tip of the cusp, sometimes reaching the arterialis layer. In 1 case verrucae were noted in the aortic pocket. Verrucae were present on the tricuspid leaflets, pockets or chordae tendineae insertions in every case. In only 3 of these were they situated on the closure line or at the tip; in 3 they were present in the pocket, and in 1 on the chordae tendineae insertions. In 1 case verrucae were present on the closure line of the pulmonic valve, and in another they were situated on the auricularis surface at about the middle of the septal leaflet. This case also showed pocket verrucae. In a 3rd case verrucae were present in the pulmonic pocket only.

As mentioned above, verrucae were noted in the aortic pocket once, in the posterior mitral pocket twice, in the tricuspid pocket three times and in the pulmonic pocket twice. Apart from these, other lesions were frequently present at this site. Thus, the great majority of cases showed a knob-like, elastified endocardial reduplication. This generally consisted of a whorled collagenous mass permeated by numerous transverse, discontinuous elastic fibers. The superficial layers of the knob were sometimes intensely elastified and were often the seat of mild or severe inflammation. Occasionally the reduplications were vascularized. An important feature was the tendency for these reduplications to involve the arterialis layer of the semilunar cusps where they shared in the thickening of the valve. In addition, polypoid formations were present in the aortic pocket in 2 cases (Fig. 8), and in the tricuspid in 1 case. The latter sometimes showed agglutination of the chordae tendineae insertions.

The chordae tendineae insertions of the anterior mitral cusp presented considerable agglutinations, with absorption into the valve tip in many instances. They also frequently possessed collagenous, sometimes inflamed vascularized reduplications. These lesions were perhaps more conspicuous in the posterior mitral cusp, where they were occasionally multiple. In one instance an Aschoff body was seen in these reduplications. Chordae tendineae absorption was not as frequently noted in the tip of the tricuspid valve. The reduplications were more delicate and less frequent. However, inflamed vascularized reduplications were noted in two instances.

As stated above, gross vascularization of the valves was frequently

observed in this group. Microscopically, vascularization or capillarization of all the valves was almost invariable. In contrast to Group I, intimal musculo-elastic hyperplastic vascular lesions were conspicuous. Inasmuch as Group I represents a clinical course whose average duration was 6 weeks, it would appear that this lesion generally requires more than 6 weeks for its development. Other observations suggest that the intimal musculo-elastic hyperplastic lesions undergo metamorphosis into intimal fibro-elastification with medial hypertrophy in less than 1 year.

Considered as a whole, the intensity and extent of the inflammatory involvement of the various valves in Group II was similar to that noted in Group I. Perhaps the most extensive involvement occurred in the aortic valve, and in this the contiguity process from the flagrant aortic ring lesion was noticeable. The thickening of the aortic cusps was often largely determined by a prolongation of thick subaortic reduplications on the ventricularis aspect of the valve and, to a lesser extent, by prolongation of aortic pocket reduplications on the arterialis side. The tricuspid lesions were severe, particularly at the root where they merged with similarly severe ring lesions. The pulmonic lesions were the least severe.

Summarizing the conspicuous features of the valvular lesions as a whole in Group II, the following points should be noted: The ring lesions were most extensive and consisted of considerable infiltration and vascularization. The blood vessels were frequently of the intimal musculo-elastic hyperplastic type. The inflammation spread in all directions. The incidence of Aschoff bodies was approximately the same as in Group I. There was considerable scarring. Practically all rings showed involvement. The highly characteristic, multiple subaortic vascularized reduplications occurred in practically every case. The intervalvular fibrosa was invariably involved.

The remainder of the valve leaflets generally showed extreme inflammation and vascularization. Many of the vessels were of the intimal musculo-elastic hyperplastic type. The valves were thicker than in Group I. This was largely due to the more conspicuous reduplications of the proximal layers which were continuous with reduplications at the valvular angles. An important contributing factor to the valve thickening was the widened, inflamed and vascularized spongiosa layer. Involvement of the fibrosa was pronounced. Aschoff bodies were present in some cases. The tips of the

valves were frequently scarred. The incidence of verrucae was higher in this group than in any other of this series. These lesions were extensive and broad; some showed beginning organization. Lesions in the pockets and on the chordae tendineae insertions were similar to Group I. Vascularization of the leaflets was practically universal.

Gross Appearance of Rheumatic Valves in Group III

(*11 Active Cases Where One Previous Attack Occurred at Least 2 Years Previous to the Fatal Outcome*)

In this group moderate or great thickening of the valve cusps was universally present in the mitral, aortic and tricuspid valves and in the majority of the pulmonic valves. Compared to the preceding groups this thickening was not only more frequent but definitely greater. Furthermore, it was less uniform, the change tending to become intensely exaggerated in the distal third of the valve cusps, particularly from the closure line to the free margin. In the latter locations, especially in the mitral valve, the cusps were enlarged to form either an exaggerated ridge or plaque. Gross vascularization was frequent.

The surface of the auriculoventricular valves showed greater deformity than in the other groups. These changes were due to rugosities, puckerings and, in 2 cases, to lime deposited diffusely throughout the mitral valve. The cusps of the auriculoventricular valves, particularly the anterior mitral, showed a definite tendency to elongation. This elongation seemed to be due essentially to the formation of new material at the free margins of the cusp, supplemented by absorption of thickened, fused chordae tendineae. The formation of a well marked shelf (overhanging the chordae tendineae of the first order) was quite frequent in these cases. The result of the chordae tendineae absorption was to make them appear definitely shortened, thus bringing the papillary muscles much closer to the margins of the cusps. The characteristic auriculoventricular valve in this group showed greatly thickened cusps with irregular surface, shelf formation and elongation with incorporation of thickened shortened chordae. Chordae tendineae of the second and third order also showed thickening of their insertions. There was stenosis of the mitral valve in 2 cases and of the tricuspid valve in 1.

In addition to thickening, the aortic valves showed notching and considerable shortening. This was due to rolling and inversion of the free margin of the cusps toward the sinus pocket (entropion). Entropion of various degrees was found in approximately half of the cases. In the majority of cases the semilunar folds either approximated the free margin or were completely invisible. Adhesion of the commissures occurred in one-third of the cases. These generally showed the fused margins separated by a delicate slit, characteristic of the rheumatic commissural lesion.

Verrucae in various stages of healing were less frequent than in either of the preceding groups, occurring in about half the cases on the auriculoventricular valves, in 3 of the 8 cases on the aortic valves, and on one pulmonic cusp. There was less tendency for the verrucae to extend onto the chordae tendineae. In the aortic valves, in addition to the previous sites mentioned, the verrucae showed a tendency to extend from one cusp to another across the commissures (Fig. 10). Double rows of verrucae were encountered somewhat more frequently than in the previous group.

Abnormalities in valve pockets were present in all cases. In 1 (tricuspid) there were yellowish masses suggestive of healed verrucae. Whitish nodules, ridges and folds were frequently present. The lining endocardium was often whitened and thickened. In 1 case there was lime in the pocket of the posterior mitral cusp. Occasionally there appeared to be healed agglutinations in the pocket of the auriculoventricular valves.

Microscopic Appearance of Rheumatic Valves in Group III

This group presented qualitative as well as quantitative differences from those previously described. As mentioned in the gross description, not infrequently most of the inflammatory processes apparently occurred with predilection toward the distal part of the cusp, generally within an area relatively confined to the closure line and tip of the valve. The inflammatory process appeared on the whole to be somewhat subdued in comparison with the findings in the first two groups. Reduplications of the auricularis layer of the mitral valve, although still frequently seen and sometimes multiple, were generally notable only at the distal end of the valve. They occurred in only one-third of the cases in the tricuspid valve. Multiple vascularized ventricularis reduplications were almost invariably

present on the aortic cusps but occurred only once in the pulmonic. They consisted of fibro-elastic strata with a tendency toward elastic-collagenous transformation. These reduplications of the proximal layers of the valves frequently fused with the spongiosa layer which was almost invariably involved. As in the previous groups, the spongiosa layer was generally considerably widened, vascularized and elastified and contained inflammatory cells, chiefly lymphocytes. Many of the vessels showed distinct hypertrophy of the media. A few cases showed hypercapillarization. Intimal musculo-elastic hyperplastic lesions were scarce.

The spongiosa layer was occasionally compressed toward the basal portion of the valves and contained distorted capillaries. While the fibrosa layer almost invariably showed involvement with capillaries or muscular vessels within the zone adjacent to the spongiosa, the inflammatory phenomena were on the whole milder than those found in the two previous groups. Elastica changes such as condensations and disruptions were quite frequent. Aschoff bodies and eosinophilic changes were rarely present. One case showed intense whorling of the intervalvular fibrosa collagen and considerable vascularization and inflammation. This showed contiguity with the aortic ring as well as with the mitral valve fibrosa.

The distal layers of the valves presented mildly inflamed reduplications in approximately half the cases. They were on the whole delicate and occasionally vascularized. The mildest lesions of the arterialis layer were found in the pulmonic cusps.

With the exception of the pulmonic cusps, the valve tips were almost invariably converted into collagenous ridges or plaques. As previously described, this was due to fusion of the proximal layers with the spongiosa layer which had undergone collagenous transformation. In the auriculoventricular valves, elastic lamellae from the fused auricularis and spongiosa layers, together with blood vessels and smooth muscle bundles, frequently curved underneath these elastic-collagenous thickenings. The tips of the anterior and posterior mitral leaflets were invariably vascularized. In some instances they were hypercapillarized.

In the gross description it was mentioned that the auriculoventricular valves, particularly the mitral, often showed considerable elongation. This was due to a fusion of the auricularis and spongiosa layers at the tip of the valve with excessive formation of elastic-

collagenous tissue. The collagenous tissue envelops or absorbs the enlarged and fused chordae tendineae insertions, in this manner prolonging the extent of the leaflets.

The tip of the aortic cusps was frequently converted into a collagenous, thickened and rounded edge which represents fusion of the spongiosa and ventricularis layers. On cross-section this formed a knob which occupied almost the entire width of the valve tip and compressed the arterialis layer. The knob itself consisted of radiating fan shaped collagenous bundles whose focal point was situated just below the tip of the valve on its arterial aspect. This focal point not infrequently showed wide, delicate walled vascular channels, probably veins.

In the tricuspid valve ridge formations were inconspicuous, even though the tip was almost invariably collagenous. Knob formation was seen only once in the pulmonic cusp tips.

The verrucae still showing eosinophilic material presented on the whole considerably less reaction at the base than those previously described. Furthermore, their incidence was lower in this group. Thus, the anterior mitral leaflet showed verrucae in 5 cases; in 4 of these they were at the closure line and showed some organization. In 1 additional case they were present on the chordae tendineae insertions. In 5 cases verrucae were noted on the closure line of the posterior mitral leaflet. In most instances these were undergoing organization. An additional case showed verrucae in the valve pocket and one on the chordae tendineae insertions. Four cases showed verrucae on the aortic valve. These were situated on the closure line and presented various stages of organization. In 2 of these cases fresh verrucae were also present in the aortic pocket. Flat organizing verrucae were present on the closure line of the tricuspid valve in 3 cases. In 2 additional cases there were verrucae in the tricuspid pocket, and in 2 others on the chordae tendineae insertions. Thus, the tricuspid valve (leaflets, pockets or chordae tendineae insertions) showed verrucae more frequently than any other valve in this group. In 2 cases verrucae were present on the closure line of the pulmonic cusps and in 2 additional cases in the pulmonic pockets.

As mentioned above, verrucae were found in the valve pockets in this group with the following frequency: in the aortic, twice; posterior mitral, once; tricuspid, twice; and pulmonic, twice. In addi-

tion 5 cases showed polypoid formations in the aortic pocket, 1 in the posterior mitral and 2 in the pulmonic. Practically every pocket showed an elastified reduplication. In some instances these reduplications formed elastified knobs such as described in Group II. In several cases the pocket reduplications were vascularized and mildly inflamed.

The incidence of verrucae on the chordae tendineae insertions has already been mentioned. Reduplications around these insertions were not infrequently seen, particularly in the mitral valve. The reduplications were occasionally vascularized or showed eosinophilic degeneration. In the anterior mitral leaflet, as noted above, the chordae tendineae were occasionally absorbed into the excessively collagenized tip. This absorption was somewhat less frequent in the posterior mitral leaflet and still less in the tricuspid. A point of interest is the fact that in this group, as well as in those subsequently to be described, the spongiosa layer of the valve leaflet opposite the chordae tendineae insertions was not infrequently widened into triangular areas containing many blood vessels.

Vascularization of the valves was invariably present in the anterior and posterior mitral leaflets, as well as in the tricuspid valve. It occurred somewhat less frequently (8 out of the 11 cases) in the aortic valve and only twice in the pulmonic. However, in this group as in those previously described, the rings showed vascularization almost invariably.

This group is the first of the series in which the clinical phenomena were of a more protracted type and the inflammatory processes, therefore, somewhat more indolent. Under such circumstances, as will be more clearly seen in the groups subsequently to be described, contiguity lesions from the ring were not as obvious as in Groups I and II. With a diminution in the intensity of the ring lesions, the inflammatory process either remained confined to the base of the valve or involved chiefly the distal third of the valve, leaving the intervening portions relatively less affected. Thus, while the inflammatory lesion in the tricuspid valve still showed contiguity from the ring and was at times quite intense, it was generally confined to the basal portion. In the pulmonic cusp the lesions were least pronounced and even more frequently confined to the ring. On the other hand, in the mitral and aortic valves the most notable lesions

were at their distal extremities. The significance of these findings will be discussed subsequently.

Summarizing the conspicuous features of the valvular lesions as a whole in Group III, the following points should be noted: The ring lesions were somewhat milder than those in the preceding groups. The vascular lesions consisted of capillaries and muscular vessels in about equal proportions. In some cases vascularization was by means of capillaries only. The incidence of Aschoff bodies was lower than in the previous groups. Intimal musculo-elastic hyperplastic lesions were infrequent. Practically all rings showed involvement. The subaortic angle invariably presented reduplications. In most instances these were multiple vascularized but less conspicuous than in Group II. Group III possessed the highest incidence of subpulmonic lesions, *i.e.*, in approximately half the cases. The intervalvular fibrosa was invariably involved.

The remainder of the valve leaflets generally showed inflammatory infiltration and vascularization of the type found in the ring. Intimal musculo-elastic hyperplastic lesions were severe. Capillaries were sometimes distorted, due to scarring. Auricularis reduplications at the base of the auriculo-ventricular valves were thinner than in Group II. The thickening of the valve was frequently confined to the tip and produced knob formation in the aortic valve. Chiefly in the mitral valve the fibrotic thickened tip was prolonged over the chordae tendineae insertions. The fibrosa layer was invariably involved, but the lesions were generally milder than in the previous groups. Aschoff bodies were infrequent. The verrucae showed a somewhat less reactive base, and many were organizing. Their incidence was lower than in the first two groups. The incidence of pocket lesions as a whole was also somewhat lower than in the previous groups. However, the incidence of aortic pocket polypi was higher than in any of the six groups in this series. This occurred in 5 of the 11 cases. The chordae tendineae insertions frequently showed considerable thickening and absorption into the valve tips. Vascularization of the leaflets exclusive of the ring was invariable in the mitral and tricuspid valves. It occurred in the aortic valve in 8 cases and in the pulmonic in 2. Several cases in this group showed lime formation at the base of the valve.

Gross Appearance of Rheumatic Valves in Group IV

(*13 Active Cases Where Repeated Attacks Took Place, Death Occurring During an Acute Recurrence*)

Diffuse thickening of the cusps of approximately the same degree as in Group III was universally present in this group. The presence of a pronounced overhanging shelf was observed on the mitral and tricuspid valves in about two-thirds of the cases. Elongation of the auriculoventricular valves, irregularity of the valve surface, and absorption of thickened chordae were present to about the same degree as in the preceding group. The shortening, thickening and fusion of ham shaped chordae tendineae insertions (Fig. 11) were more advanced than in the preceding group. There were occasional agglutinations in the pockets formed by the chordae attachments. Lime was present only in one mitral valve.

The alterations of the aortic valve (Fig. 10) were quite similar to those in the preceding group, being characterized by thickening and shortening of the cusps, approximation of the semilunar folds to the free margin, or their disappearance, rolling, inversion and notching of the free margin, and commissural agglutination.

Fresh and healing verrucae were present in the great majority of all of the valves. Their occurrence, particularly in the tricuspid and pulmonic valves, was much more frequent than in Group III, being present in almost every case. Double rows were seen in a few instances.

Vascularization, chiefly of the mitral valve, occurred with considerable frequency.

Pocket lesions were present in all cases. The pocket angles were generally widened and irregular. The endocardial lining was white or gray and thickened. Yellowish and whitish smooth nodular elevations were often present. Transverse and radial ridges and folds occasionally occurred. Agglutinated verrucae were found in the pocket of one posterior mitral cusp.

Microscopic Appearance of Rheumatic Valves in Group IV

The microscopic lesions of the valves in this group were, on the whole, somewhat similar to those described in Group III. There were, however, several interesting differences which were undoubtedly a reflection of the clinical course (repeated attacks). It was

previously shown that vascular lesions of the intimal musculo-elastic hyperplastic type probably require more than 6 weeks for their development. Their scarcity in Group III indicates that these lesions undergo fibro-elastic metamorphosis after 2 years. (Other observations suggest that such involution may occur in less than 1 year.) Inasmuch as Group IV represents repeated attacks, one of which might have occurred within 1 year before death (thus simulating Group II), it is not surprising that many of the vascular lesions in Group IV were of the intimal musculo-elastic hyperplastic type. However, these were not nearly as conspicuous or as frequently present as in Group II, capillaries and muscular vessels being the predominant type of vascularization. Another interesting feature was the much more frequent occurrence of the collagenous thickening of the valve tips. This will be discussed in greater detail later.

Reduplications of the proximal layers of the valves were generally present. These were frequently delicate in the proximal two-thirds of the leaflets, particularly in the auriculoventricular valves. In this thinner portion of the valve leaflet, vascularization was frequent and often quite superficial. The spongiosa was widened, vascularized, elastified and in general moderately inflamed, particularly in the anterior and posterior mitral leaflets. In the mitral valve the widened spongiosa layer frequently contained large smooth muscle bundles in apposition to the auricularis layer. In the tricuspid valve the spongiosa showed its widening chiefly in the triangular zones above the chordae tendineae insertions.

The fibrosa layer of the auriculoventricular valves almost invariably showed moderate inflammation with some elastification and elastica distortion. The fibrosa layer of the aortic valve showed little involvement. Most of the cases presented intercryptic swollen cells in the fibrosa of the pulmonic cusps. These cells were generally less abundant in cytoplasm than were those found in the previous groups. In 1 case there were whorling and vascular permeation of the fibrosa collagen in the anterior mitral leaflet. This inflammatory lesion could be traced through the intervalvular fibrosa to the aortic annulus. Aschoff bodies were observed in only one valve. This was in the aortic fibrosa. On the whole it may be said that the fibrosa lesions in this group were the mildest of those thus far described, but they were still present in the majority of instances, except in the aortic cusps.

The ventricularis layer of the auriculoventricular cusps showed delicate collagenous reduplications. In a few instances these were vascularized and presented mild inflammatory changes. Similar changes occurred in the arterialis layer of the semilunar cusps. Inflamed arterialis reduplications occurred more frequently in the aortic than in the pulmonic cusps.

In almost every case each valve tip, except the pulmonic, showed fibrosis. The tips of the anterior and posterior mitral leaflets were thickened, elastified, collagenous and vascularized. As in previous groups, this was due to fusion of the collagenous spongiosa and auricularis layers. Redundant collagen from these fused and thickened valve tips frequently spread for a considerable distance over the chordae tendineae insertions and produced elongation of the cusps. Deviation of the auricularis and spongiosa elastic lamellae, blood vessels and smooth muscle bundles were generally noted beneath the thickened fused mass at the tip. On cross-section the tips of the aortic valves were represented by large collagenous knobs in every case (Fig. 12). Histologically these were similar to those described in Group III. The pulmonic cusps were the least involved and showed knobs only occasionally. The tricuspid valve tips were generally collagenous and sometimes showed extension over the chordae tendineae, but extensive thickening was infrequent.

The verrucae present in this group were generally of a more indolent type than those found in the previous groups. Many of these were flat, on a broad base and with little reaction in the underlying tissue. A number of them showed considerable organization and absorption within the leaflet. In a few cases the verrucae appeared to represent merely an eosinophilic degeneration of the superficial collagenous layer of the valve leaflet, chiefly at the closure line or around the tip of the valve.

The anterior mitral cusp showed fresh verrucae in 6 cases. These were generally situated at the closure line or near the tip. Some were undergoing organization. In 1 of these cases verrucae were also found on the chordae tendineae insertions. In 9 cases the posterior mitral cusp showed verrucae. Most of them were organizing. Verrucous lesions were present in one of the posterior mitral pockets. No verrucae were found on the chordae tendineae insertions of this cusp. In 7 cases verrucae were present on the aortic cusp. These were situated either on the closure line or around the tip. They were

generally broad and showed relatively little reaction. In 1 case verrucae were also present in the aortic pocket. Ten of the cases showed verrucae on the tricuspid valve, an unexpectedly high incidence, and the highest in this group. These were generally broad also, many resembling eosinophilic change. Some were undergoing organization. Three of the cases showed verrucae in the tricuspid pocket and 3 on the chordae tendineae insertions. Thus, in 12 of the 13 cases in this group, fresh verrucae were present on some portion of the tricuspid valve. Chordae tendineae agglutinations in the tricuspid pocket were also sometimes seen. Obviously, therefore, the tricuspid valve lesion, although leading to less fibrosis than the other cusps, maintains activity in an extraordinarily high percentage of cases. In 5 of the cases verrucae were present on the pulmonic cusps. These also were generally flat or organizing. Two cases showed verrucae in the pocket. In 6 of the 13 cases in this group verrucae were present on some portion of the pulmonic valve. This represents the highest incidence of pulmonic valve verrucae of any group and is, undoubtedly, a reflection of the multiple attacks.

As mentioned above, verrucous lesions were found in the valve pockets with the following frequency: once in the posterior mitral, once in the aortic, three times in the tricuspid and twice in the pulmonic. In addition, the aortic pocket contained polypoid lesions in 3 cases, the posterior mitral in 2, and the pulmonic in 1. Most of the cases showed elastified distorted pocket reduplications, some of which formed large knobs. In a few cases vascularized reduplications were present in the pockets.

The incidence of verrucae on the chordae tendineae insertions has already been referred to. As noted, absorption within the mitral leaflet tip was almost invariably present. Multiple vascularized inflamed reduplications were present on some chordae tendineae insertions of the mitral valve. In a number of cases many of the chordae tendineae insertions of the tricuspid valve also showed absorption.

Vascularization was present grossly and microscopically in almost all the cusps except the pulmonic, where it was noted in 6 of the 13 cases in this group. Ring lesions with vascularization were almost invariably present.

Even though the inflammatory lesions on the whole were not as varied in Group IV as in the other groups described, there were

present other manifestations of continued damage which placed this group after the first two in order of activity. On the other hand, because of the somewhat prolonged course and repeated attacks, there were present also evidences of chronicity which approximated the changes present in the groups subsequently to be described. It is, therefore, this combination of fairly active lesions and extensive healing which characterizes this group. The greatest distortion and thickening of the valve leaflets occurred in the posterior mitral cusp. Of interest was the fact that the tricuspid valve was still consistent in showing notable exudative lesions. Inflammatory involvement of the pulmonic valve was not infrequently most pronounced in the middle portion of the cusps. This is of considerable interest as indicating the tendency for lesions in this valve to be arrested before spreading to the tip.

Summarizing the conspicuous features of the valvular lesions as a whole in Group IV, the following points should be noted: The ring lesions consisted only of distorted capillaries caused by the scarring process. The occurrence of inflammatory cells was less frequent than in the previous groups. Aschoff bodies were most infrequent. All the rings were involved. All the subaortic angles showed lesions that were almost invariably of the multiple elastified variety. These, however, were not as great as in the first two groups. The intravalvular fibrosa showed a high incidence of lesions (11 of 13 cases).

The remainder of the valve leaflets generally showed somewhat milder exudative phenomena than in Group III. Vascularization was similar to Group III but, in addition, intimal musculo-elastic hyperplastic vessels were more frequent. Because of more definite scarring, distorted capillaries were frequently seen. In some instances the proximal two-thirds of the valve leaflets were fairly thin and showed superficial vascularization with thick vessels. In others the valve was diffusely thickened. Marked fibrosis and thickening of the tips of the mitral, aortic and tricuspid valves were noted more frequently in this group than in the others thus far described. The most notable thickening was that in the posterior mitral leaflet. This thickening of the tips of the auriculoventricular valves, together with elongation of the leaflets, was due to the same process as previously described. The fibrosa of the auriculoventricular valves almost invariably showed a mild degree of inflammatory involvement.

The aortic fibrosa was generally intact. That of the pulmonic valve showed intercryptic cells with basophilic cytoplasm. Aschoff bodies were found in the fibrosa only once.

The verrucae in this group showed even more indolence than in Group III. A number of them consisted of eosinophilic, swollen and degenerated collagen with little reaction at the base. On the other hand, the incidence of verrucae in this group was higher than in Group III, though somewhat lower than in the first two groups. The incidence of polypoid and verrucous pocket lesions also placed this group third in order of frequency. The incidence of verrucae on the chordae tendineae insertions and the absorption of the latter into the valve tip were somewhat similar to Group III. Vascularization of the valves was extremely frequent, occurring almost invariably in every valve except the pulmonic, where it was noted in 6 of the 13 cases in this group.

Gross Appearance of Rheumatic Valves in Group V

(28 Active Cases Where Death Was Caused by Decompensation Without Clinical Evidence of a Final Acute Attack. Some of These Cases Had No Previous History of Rheumatic Fever)

The cases in this group showed the most advanced alterations of any group. Definite diffuse thickening of the cusps was universally present in the mitral, aortic and tricuspid valves, and there was moderate thickening of the pulmonic valve. The formation of a ridge or thickening in the peripheral portion of the mitral cusps was more frequent than in the preceding group, being present in half the cases. A pronounced overhanging shelf also was present on the mitral valve in half the cases. In 9 cases the valve was diffusely infiltrated with lime which notably distorted the cusps by its projection through the auricular and ventricular surfaces. In a number of instances vertical cracks appeared through the lime at the commissural regions. Buttonhole stenosis of the mitral valve was present in 4 cases.

As in the preceding group, the aortic valves were greatly thickened, shortened, and their edges rolled and inverted. Notching was most frequent. There was a much greater tendency to the deposition of lime in the cusps themselves, in the region of the noduli, and in the commissures. Adhesions of the cusps at the commissural margins was much more frequent than in the preceding group. The

valves in general appeared much more distorted and were rigid, a characteristic not found in the preceding group.

Fresh and organized verrucae were less frequent than in the preceding group, being present in 6 cases on the mitral valve, in 10 on the aortic, in 12 on the tricuspid and once on the pulmonic valve. Double rows of verrucae were observed in several cases. Thickening, fusion, absorption and shortening of the chordae tendineae were more pronounced than in the preceding groups. Generally, only the valvular attachments of the chordae tendineae of the third order were still present. Not infrequently the papillary muscles were almost in contact with the valve margins.

Gross vascularization occurred with considerable frequency and was found chiefly in the mitral, aortic and tricuspid valves.

The pockets were characterized chiefly by a whitening and thickening of the endocardium; there was a tendency for the auriculoventricular valve leaflets to form agglutinations with the ventricular wall and thus obliterate the sharp pocket angle. Further irregularities and obliteration of the auriculoventricular pocket angles were due to the frequent presence of fibrous bands and muscular bridges at this site. The pockets of the aortic cusps and, to a less extent, of the pulmonic cusps, frequently contained nodules, ridges and folds.

Microscopic Appearance of Rheumatic Valves in Group V

The auricularis layer of the auriculoventricular valves as well as the ventricularis layer of the aortic valve showed multiple elastified reduplications in approximately half the cases. These were frequently quite thick and contained sparse scatterings of lymphocytes. Fusion of the elastic-collagenous terminations of these layers with the similarly transformed tip of the spongiosa layer produced considerable thickening. The reduplications on the proximal valvular surfaces were all vascularized in the tricuspid valve, and generally in the posterior mitral leaflet and aortic valve. Only a few cases showed vascularization of the auricularis layer in the anterior mitral leaflet. In the pulmonic cusps the ventricularis layer was either intact or showed more delicate reduplications, only a few of which were vascularized. The spongiosa layer of the valves was almost invariably thickened and vascularized, and generally mildly inflamed. The vascularization, particularly in the spongiosa layer, consisted of greatly hypertrophied muscular vessels, sometimes of the intimal

musculo-elastic hyperplastic type. In a few instances the collagenous transformation of the auricularis layer produced compression of the spongiosa layer.

Although 1 case showed definite whorling and vascularization of the fibrosa collagen, this layer was much less frequently involved than in the previous groups. In this respect Group V differed greatly from the preceding groups. Furthermore, in this group lipoid and calcific deposits involving the spongiosa as well as the fibrosa layers occurred considerably more frequently. It is to be noted, however, that the average age period of this group was somewhat older than the preceding. Aschoff bodies were seen only once. These were present in the anterior mitral leaflet. The fibrosa layer of the pulmonic valve generally showed intercryptic cells.

Most of the auriculoventricular valves showed delicate collagenous ventricularis reduplications. These were considerably exaggerated in width at the site of the chordae tendineae insertions, and on microscopic section presented the appearance of conspicuous crescents. In many instances, particularly on the mitral valve, the ventricularis reduplications were vascularized. In several instances the arterialis layer of the aortic valve consisted of widened collagenous extensions of pocket reduplications. These showed moderate inflammation, sometimes vascularization, and extended as far as the tip of the cusp, thus increasing the thickness of the leaflet. In several cases the arterialis reduplications were enormously thick and were associated with entropion of the aortic valve tip. The arterialis and ventricularis layers of the pulmonic valves were either uninvolved or showed delicate reduplications.

The tips of the mitral (Fig. 13), aortic and tricuspid valves were practically all converted into elastic-collagenous masses. These thickened tips were almost invariably vascularized and showed elastic bands and smooth muscle curving under the fused auricularis and spongiosa terminations. Excessive collagen formation with absorption of fused chordae tendineae insertions and elongation of the cusps was frequent in the auriculoventricular valves (Figs. 14 and 15). Some leaflets showed a moderate degree of inflammatory reaction. The tip of the pulmonic valve frequently showed fibrosis but the formation of knob-like thickening was seen in only one instance.

In spite of the fact that in the cases in this group death took place from decompensation without clinical evidence of a final acute at-

tack of rheumatic fever, and that some of these had no previous history of this condition, approximately 20 per cent of all the valve leaflets showed verrucae. This, together with the invariable presence of Aschoff bodies in the myocardium of these cases, as well as the mild inflammatory lesions in the cusps and elsewhere, indicates that decompensation in rheumatic valvular disease is frequently an evidence of activity of the rheumatic process, as contended by Rothschild, Kugel and Gross,[48] and others.

In the majority of instances the verrucae in this group were broad, flat, extremely indolent, showed little or no reaction at the base and presented the appearance of eosinophilic collagen degeneration of the superficial layers of the valve. A number of verrucae showed advanced organization. Completely organized verrucae were represented by ridges of proliferated fibroblasts at various levels on the valve tips. In 5 cases fresh or organized verrucae were situated either on the closure line or at the tip of the anterior mitral leaflet. In 2 of these the lesions resembled eosinophilic collagenous degeneration. An additional case showed a similar process involving the insertions of the chordae tendineae. In 6 cases organizing verrucae were present on the closure line or at the tip of the posterior mitral cusp. In 1 case these were also present on the chordae tendineae insertions. In 6 cases the aortic cusp showed fresh or organizing verrucae on the closure line or tip. Most of these were of the nature of an eosinophilic swelling and degeneration. In 7 cases flat verrucae or eosinophilic collagenous degeneration were present on the closure line of the tricuspid valve. One of these cases also showed verrucae on the chordae tendineae insertions. In 2 cases there were indolent verrucae on the closure line of the pulmonic cusps.

None of the valve pockets in this group showed verrucae. On the other hand, scarring of the underlying annulus and delicate elastified reduplications were present in all the valve pockets. The reduplications were inflamed and vascularized in the posterior mitral pocket in 4 cases and in the tricuspid valve pocket in 6 cases. Occasionally the pocket reduplications were multiple. The pulmonic pocket was the least involved. Besides these lesions, one aortic pocket showed a polypoid structure. Deposition of lipoid crystals in the pockets, especially toward the later age periods, was occasionally seen.

As mentioned above, the chordae tendineae insertions of the mitral and tricuspid leaflets showed verrucae in a few cases. In

several instances the reduplications were multiple and vascularized. In many cases the chordae tendineae insertions were absorbed into the elastic-collagenous valve tip and incorporated into the collagenous extension of this structure with resulting elongation of the cusps. Agglutination of the chordae tendineae to the endocardium was frequently noted in the tricuspid pocket.

Gross and microscopic vascularization was almost invariably noted in the mitral and tricuspid valves. Only 17 of the 28 cases in this group showed vascularization of the aortic valve. Vascularization of the ring was present in these and in 4 additional cases. In 13 cases the pulmonic valve showed vascularization. Vascularization of the ring was present in these and in 6 additional cases. Thus far, therefore, in the five groups described, universal vascularization of either valve leaflets or rings was found in the great majority of cases.

Inflammatory phenomena were extremely indolent in this group. However, when activity was present it still appeared to be most noticeable in the tricuspid leaflet. In a number of cases the exudative phenomena seemed to stop at the rings. Although vascularization was not present in the various valve leaflets of a number of cases, the proximal valve layer and the spongiosa layer not infrequently showed considerable thickening with transformation into collagenous ridges. Indeed, some of these cases showed indolent verrucae and one presented Aschoff bodies. This suggests the possibility that a low grade toxic process proceeding from the ring was able to elicit this gradual inflammatory transformation of the valve leaflet, chiefly at its distal portion, but was insufficient to stimulate the formation of blood vessels.

Not infrequently the posterior mitral valve was the one to show the greatest thickening process. This was often confined to the tip of the valve, the proximal part showing little more than superficial vascularization involving only the auricularis layer.

A number of the valve leaflets which were intact in other respects showed elastica condensations and ruptures in the spongiosa layer. In many cases the pulmonic cusps were notable for the complete absence of inflammatory cells. However, the relatively high incidence of vascularization in this valve indicated a previous inflammatory process.

Summarizing the conspicuous features of the valvular lesions as a

whole in Group V, the following points should be noted: The ring lesions showed considerable diminution in their extent, intensity and incidence. The blood vessels were generally thick walled arterioles or arteries, or distorted capillaries. The rings showed scarring and elastica distortion. Cellularity was sparse and Aschoff bodies rare. Subaortic lesions occurred in approximately half the cases. These were generally of the multiple vascularized variety. The incidence of the intervalvular fibrosa lesion was approximately 50 per cent. This was generally mild. In contrast with the previous four groups, universal ring lesions occurred in approximately half the cases. Ring lesions were found in three rings in another 25 per cent of the cases. Every case showed involvement of at least two rings.

The remainder of the valve lesions generally showed extremely mild exudative phenomena. Vascularization consisted of thick walled vessels and of capillaries, often distorted by scar tissue. Intimal musculo-elastic hyperplastic vessels were scarce. Thickening of the valve leaflet as a whole, due to somewhat heavier reduplications, was more frequently seen than in the last group. Thickening and elastification of the valve tips was also more frequent. On the other hand, the incidence of relatively intact leaflets was higher in this group than in the preceding one. As in Group III, the posterior mitral leaflet frequently showed the greatest thickening, and the tricuspid valve the greatest activity. Deformity of the aortic valve was often due to enormously thickened ventricularis and arterialis layers. The fibrosa layer was much less frequently involved than in the preceding groups. On the other hand, lime deposits in this as well as in the spongiosa layer occurred with considerable frequency. Aschoff bodies were found in 1 case. Verrucae occurred in approximately 20 per cent of the cases, the lowest incidence of any of the groups thus far described. These lesions appeared most frequently in the tricuspid valve and, in most instances, consisted of eosinophilic swelling and degeneration of the superficial layers of the leaflet at the tip. Polypoid lesions were found in only 1 case. This was in the aortic pocket. No pocket verrucae were present in this group. The chordae tendineae insertions showed crescentic reduplications in many cases and were often greatly thickened. Even though vascularization of the mitral and tricuspid valves was almost invariable, the incidence of vascularization of the

aortic valve decreased considerably (17 of 28 cases). The pulmonic valve was vascularized in 13 cases.

Gross Appearance of Rheumatic Valves in Group VI
(*26 Inactive Cases of Chronic Valvular Disease of the Typical Rheumatic Variety*)

The changes in this group varied considerably in severity. Some of the cases showed the extreme valvular deformities that have been described in Group V. There were many instances, however, in which one or more of the valves was either entirely normal or showed only a mild or moderate thickening. In selecting the cases for this group there were excluded those that presented the generally large secondary thrombotic verrucous lesions which have been separately described by Gross and Friedberg [52] under the classification of non-bacterial thrombotic endocarditis.

The mitral valve generally showed distinct thickening. Healed verrucae were present in only 4 cases out of the 26. Vascularization was visible in 14 cases. In 9 the terminal portion of the valve was converted into a collagenous mass. Lime was present in 6 cases, and in 2 there was definite stenosis of the valvular surface. In only one-fifth of the cases was there extreme deformity of the valve with elongation and absorption of thickened, shortened chordae tendineae.

Half of the cases showed only a mild or moderate thickening of the aortic valve. Healed verrucae were present in 1 case. In 4 cases there was a shortening of the cusp with notching, rolling and inversion of the margin, as previously described. In about one-fourth of the cases the semilunar folds approximated the free edge, or were obliterated. In 3 cases the cusps were rigid, chiefly because of infiltration of lime. Adhesion of the cusps at their commissures was present in 8 cases.

Most of the tricuspid valves showed only a mild or moderate diffuse thickening. Healed verrucae were present on three of the valves. Occasionally there was a loss of normal scalloping or an early shelf formation. In a few cases there was shortening and thickening of the chordae tendineae. The pulmonic cusps showed a mild degree of thickening in about two-thirds of the cases. In the remaining cases the valve was normal.

Abnormalities in the valvular pockets were present in about two-thirds of the cases. These abnormalities were qualitatively similar

to those in the preceding group, consisting of whitening and thickening of the endocardial lining, the presence of ridges, tiny nodules, folds and muscular and fibrous bridges which distorted the regularity of the pocket angle.

Microscopic Changes of Rheumatic Valves in Group VI

Practically all the mitral valves in this group showed collagenous auricularis reduplications which were generally quite flat in the proximal two-thirds of the valve, and possessed superficial vascularization with muscular vessels. In many cases the tip of the valve was converted into a collagenous mass. About half of these thickened tips were vascularized. Practically every case showed reduplications of the aortic valve ventricularis. In one-third of the cases these were multiple, and in one-half the cases they possessed enormously thick vessels with narrowed lumens. The lesions of the tricuspid valve were generally much less striking. The pulmonic valve generally showed either a delicate collagenous reduplication of the ventricularis layer or no discernible lesion.

The only consistent spongiosa lesion found in this group was in the posterior mitral cusp. In half the cases this layer was widened, elastified and vascularized. The widening was particularly noticeable over the chordae tendineae insertions. The blood vessels in the spongiosa layer were generally greatly hypertrophied and often showed intimal narrowing. Sparse scatterings of mast cells and lymphocytes were occasionally noted. In the tricuspid and aortic valves, vascularization of the spongiosa layer was infrequent. In the pulmonic valve it was rare. Chiefly in the mitral valve, the spongiosa sometimes showed considerable compression by the collagenous auricularis.

Apart from degenerative changes (hyaline transformation or lipoid or calcific deposition), the fibrosa layer was practically uninvolved in these cases. Occasionally it was thinned. In 1 case this layer showed whorling of the collagen and vascularization.

In 3 cases the ventricularis layer of the anterior mitral leaflet showed multiple reduplications. In 4 they were vascularized and richly elastic-collagenous. Apart from these, about half the cases showed crescentic reduplications of the chordae tendineae insertions. In the posterior mitral leaflet and in the tricuspid valves the ventricularis layer showed little else than crescentic thickenings of

the chordae tendineae insertions. In half the cases the aortic valve arterialis showed collagenous reduplications. The arterialis of the pulmonic valve was practically intact.

The tips of the mitral valve were invariably converted into elastic-collagenous thickenings. In two-thirds of the cases these thickened tips were vascularized. The tricuspid valve showed considerably less thickening of the tip. On the other hand, two-thirds of the cases showed extreme collagenous thickening of the aortic valve tips. These were less frequently vascularized than the mitral and generally consisted of the fan shaped collagenous knob structure described above. The tips of the pulmonic cusps showed no collagenous thickenings or other changes.

The only lesions in any way resembling verrucae found in this group were eosinophilic swelling and degeneration occurring generally on the closure line and showing practically no reaction of the underlying structure. These lesions were, on the whole, delicate and were found once on the anterior mitral leaflet, once on the posterior mitral, three times on the aortic and once on the tricuspid.

No verrucae or polypoid lesions were found in the valve pockets. On the other hand, all the valve pockets showed elastified reduplications. These were generally quite delicate and, in a few instances, vascularized. No verrucae were found on the chordae tendineae insertions. These frequently showed absorption into the mitral valve tip. In about half the cases the chordae tendineae insertions into the mitral and tricuspid valves showed crescentic reduplications. In 1 case the reduplications were vascularized.

The incidence of vascularization of the valves as a whole showed a conspicuous difference from the previous groups. Thus, while the anterior mitral leaflet was almost invariably vascularized, capillaries or muscular vessels were present in 20 of the 26 cases in the posterior mitral leaflet, in 12 cases in the aortic, in 8 cases in the tricuspid and only in 4 cases in the pulmonic. In 9 additional cases vascularization was present in the aortic ring, in 9 in the tricuspid ring and in 11 in the pulmonic ring.

Considered as a whole, this group showed a considerably lower incidence and severity of valvular deformities. Reduplications of the proximal valve layers were either delicate or not present. The mitral valve showed the most advanced lesions. Vascularization, when present, was frequently quite superficial on the proximal two-thirds

of the leaflets. Angle lesions, both over the auriculoventricular and semilunar rings were inconspicuous. Although the tricuspid valve was practically free from exudative phenomena, capillaries were still conspicuous. The pulmonic valve was notable for the frequency with which it was intact and for the slight extent of the deformity when it was involved.

Summarizing the conspicuous features of the valvular lesions as a whole in Group VI, the following points should be noted: The ring lesions showed practically no exudative phenomena. Aschoff bodies were not present. Inflammatory cells were extraordinarily sparse, scarring was appreciable and vascularization consisted of capillaries, hyaline arterioles or hypertrophied vessels. Subaortic lesions occurred in approximately half the cases. Half of these were multiple elastified reduplications and only half of these again were vascularized. Only 1 case of the 26 in this group showed a subpulmonic lesion. Intervalvular fibrosa lesions were found in only 5 of the cases. These were extremely mild. Only 6 cases showed universal ring involvement and in another 7, three rings were involved. Every case showed involvement of at least one ring.

The remainder of the valve leaflets was equally free of exudative phenomena. Vascularization was by means of capillaries and extremely thick walled vessels with greatly narrowed lumens. These were usually found in the superficial layers of the valve. Many valves were completely intact or showed some elastification of the fibrosa layer. Thickening of the leaflets occurred chiefly in the mitral valve and usually affected the tip in a manner similar to that described for the previous two groups. The only fibrosa lesion present was deposition of lime, which occurred in several cases. No Aschoff bodies were present. The only changes approximating verrucae were found in but few valves. These consisted of eosinophilic degeneration of a completely bland type. The pockets generally showed delicate reduplications and were free from verrucae or polypoid lesions. When involved, the chordae tendineae insertions showed reduplications generally of the crescentic variety. The anterior mitral leaflet was the only one which was almost universally vascularized. Blood vessels were found in 20 of the 26 cases in the posterior mitral leaflet, in 12 in the aortic, and in 4 in the pulmonic.

Discussion

In a previous report [46] it was shown that the rings are likely to be the first parts of the valves involved in a rheumatic affection. Briefly considered, the observations which support this view are as follows: (1) the valve rings almost invariably show inflammatory changes even when the remainder of the valve leaflets is relatively free from disease; (2) the lesions in the rings are generally more flagrant than those in the rest of the leaflets; and (3) particularly in the active cases, a definite contiguity inflammatory process can usually be seen extending from the valve ring into the body of the leaflet.

In the same report a discussion was given of the mechanisms concerned with the localization of the inflammatory process in the valve rings and with the spread of these lesions to and from the several rings. It appears that three mechanisms may play a rôle in this connection: (1) the simultaneous involvement of all the valve rings by the spread of infection from the adjacent myocardium; (2) involvement of the aortic and pulmonic rings by spread from the roots of the great vessels; and (3) involvement of the mitral ring from the inflamed left auricle with contiguity spread to the other rings, chiefly by way of the annulus extensions, but also through the pericardial wedges. It was shown that blood vessels do not exist in most normal rings and that they are probably not concerned with the initial localization of the rheumatic lesions in these sites.

The observations incorporated in the present report afford an excellent opportunity to study the pathogenesis of the valvular lesions and their life cycles. Assuming that the initial valvular lesion is in the ring, it appears that spread of the infection takes place by a contiguity process chiefly through the spongiosa layer, as well as the proximal layers of the valves (auricularis layer of the auriculoventricular valves and ventricularis layer of the semilunar valves). The spongiosa layer undergoes considerable widening, with edema and inflammatory infiltration. The proximal layers of the auriculoventricular and semilunar valves are prone to the formation of reduplications whose structure is somewhat similar to that previously described in the left auricle (Gross [43]). Thus, the generalized valvular thickening which occurs during an initial attack or an acute exacerbation is due partly to extensive exudation and partly to the formation of these reduplications of the proximal layer of the valves. It

appears further that verrucae result from certain exudative and degenerative processes which occur on the proximal surface of the inflamed valves, chiefly at the closure line. These processes are frequently associated with proliferation of the local fixed cells. These often arrange themselves at right angles to the surface and form the so-called palisades. This, therefore, is not a primary lesion as believed by Leary,[34] but occurs subsequent to the valvulitis. The secondary rôle in the thickening process played by the formation of verrucae will be considered separately.

In the more chronic stages, deformity of the auriculoventricular valves is produced by thickening of these reduplications, formation of multiple reduplications, elastic-collagenous transformation of the tips of the leaflets and, in some cases, lipoid and calcific deposition in the annulus around the pocket, the fibrosa layer and the thickened tip. If the lesion is of long duration there may be added to these, thick reduplications on the ventricularis layer. Together with these processes other changes generally occur. Thus, fibrosis of the auriculoventricular valve tips is frequently associated with excessive collagen formation, part of which results from organization of verrucae. In the auriculoventricular valves this collagen spreads over and fuses with the chordae tendineae insertions, which are thickened by fibro-elastic reduplications, with resulting elongation of the valve leaflet. These elongated leaflets, with their incorporated chordae tendineae, are molded together by redundant cicatrizing connective tissue as a result of which there is eventually created a rigid, flattened or rounded, funnel shaped structure — the typical valvular stenosis. A similar process, in the absence of redundant connective tissue, leads to a less marked stenosis without lengthening of the valve leaflets — indeed, at times with shortening due to cicatrization. Thus, the formation of auricularis, ventricularis and chordae tendineae reduplications, the profuse collagenization of the valve tips and the fusion of the thickened chordae tendineae insertions with the thickened valve tips are responsible for the characteristic deformities of the auriculoventricular valves in chronic valvular disease. To these may be added the secondary lipoid and calcific changes and, in some cases, secondary thrombotic deposits on the closure line with organization (Gross and Friedberg [52]).

Deformity of the semilunar cusps occurring in the chronic cases is due to a somewhat similar process, modified, however, by the to-

pography of the commissures and by the absence of chordae tendineae. The close approximation of the inflamed semilunar folds between two cusps frequently leads to their agglutination. This produces the characteristic rheumatic commissural lesion in which a delicate groove persists between the agglutinated cusps. Eventually, thickening of the cusps and commissures leads to stenosis. The redundant collagen formation at the tip of the ventricularis layer, which is partly due to organization of verrucae, causes the semilunar cusps to fold over or become fused with a thickened arterialis layer (entropion). The latter, as has been shown, rarely undergoes the excessive collagen transformation seen in the ventricularis layer. In some instances the tips of the cusps fold over toward the lumen of the heart, producing ectropion. It is seen, therefore, that in contrast to the auriculoventricular valves the semilunar cusps are shortened by the infolding (or outfolding) of the tip. As a consequence, the semilunar folds on these cusps approximate the newly formed free edge and may eventually disappear completely. Furthermore, the infolding of the nodulus Arantii produces a distinct notching at the center of the free edge of each cusp.

It is clear from the above that the presence of chordae tendineae permits the redundant collagenous tissue to fuse with them and produce elongation of the auriculoventricular valves. On the other hand, their absence on the semilunar cusps causes the same inflammatory process to produce shortening and generally entropion of the semilunar valves with notching and approximation of the semilunar folds to the free edge or their disappearance. Obviously, the degree to which deformity will take place depends on the acuity of the inflammatory processes, on their repetition, on the valve affected, as well as on the type of reaction elicited in the given case. The latter plays a prominent rôle in determining the extent of the secondary lipoid and calcific changes which occur. As has been emphasized by Libman,[53] there are undoubtedly definite individual differences in the propensity for the deposition of calcific material in blood vessels, pericardial exudate, valve rings and valve leaflets.

The deposit of lime in the cusps in turn leads to characteristic gross and histological changes. It has been shown above that the fibrosa layer of the cusps becomes hyaline and relatively acellular with advancing age periods and that, simultaneously with these changes, lime deposition takes place. Apparently the same process

occurs in the definitely collagenous, hyaline and relatively acellular structure found in the scarred ring annulus, fibrosa layer at the base of the valve, and thickened valve tips. As has been shown for certain blood vessel lesions, rheumatic fever may greatly hasten a normally occurring degenerative process. It has been indicated before that these changes are apt to occur with predilection at the commissural regions. Secondary to the lime deposition, granulation tissue capillaries and inflammatory cells may surround the foreign material and lead to agglutination of the cusps. At times the calcific material in the commissures, particularly of the mitral valve, shows characteristic vertical cracks. These sites may be covered with thrombotic material and become infected with bacteria, thus producing a bacterial endocarditis. Metaplasia with bone and bone marrow formation may occur in the areas of lime deposition.

It was previously shown that the lesions in Group I (cases with death during a first attack) are characterized by the acuity and extent of the exudative phenomena and by the fact that the vascularization of the valves is mainly of the capillary type. Reasons were given which indicate that 6 weeks are generally insufficient for the production of muscular wall vessels by the rheumatic process. In the Group II cases the patients had suffered one previous attack within 1 year of the fatal outcome. In these the exudative phenomena were even more distinct. In addition, characteristic reduplications were present at the valve angles and on their surfaces, and the vessels present in the inflamed valves were frequently of the intimal musculo-elastic hyperplastic type. It appears that this type of vessel lesion, therefore, apparently generally requires more than 6 weeks for its production. In Groups III, IV and V the active exudative phenomena became increasingly indolent and were absent in Group VI. The vasculature in the valves of these more chronic groups consisted of capillaries, often distorted by scar tissue, and of vessels with muscular walls, and sometimes intimal fibrosis (particularly in Groups V and VI).

Of great interest is a consideration of the verrucous lesions found in these cases. Their incidence was unexpectedly high and apparently closely paralleled the clinical course of the disease. Thus, verrucae were almost invariably present on the mitral, aortic and tricuspid valves (including the pockets and chordae tendineae insertions) in Group II, and occurred with high frequency in these

valves in Group I. Group IV was the next in order of frequency as regards the incidence of these lesions. This was not unexpected in view of the fact that this group represents cases with repeated attacks. Furthermore, inasmuch as one of these rheumatic bouts occurred within 1 year of the fatal outcome in a number of cases (thus simulating Group II), the incidence of intimal musculo-elastic hyperplastic vascular lesions was also somewhat high in this group. Verrucae occurred in approximately 50 per cent of the valves in Group III. Their incidence fell considerably in Group V, occurring in approximately 20 per cent of the valves. In Group VI they were rare and consisted entirely of degenerated eosinophilic material resulting from collagen necrosis. The relation of these lesions to nonbacterial thrombotic endocarditis (Gross and Friedberg [52]) will be subsequently discussed. Verrucae were present on the pulmonic valve in over 40 per cent of the cases in Groups IV and II. Their incidence was somewhat lower in the pulmonic valves in Groups III and I. They were rare in Group V and not present in Group VI.

The histological structure of the verrucae suggests that they are made up of hyaline eosinophilic material resulting from necrosis and fusion of inflammatory exudate as well as of the superficial layers of the valve. Their appearance is apparently greatly influenced by the clinical course of the disease. Thus, they were fresh and showed considerable reaction at the base in Group I. In Group II they were broad and presented evidence of some organization and considerable reaction at the base. In Group III they were broad, showed increasing incidence of organization with, however, reaction still present at the base. In Group IV some verrucae appeared to consist of eosinophilic degeneration of the superficial layers of the valve with somewhat milder reaction at the base. In Group V the great majority of verrucous lesions consisted of extremely indolent lesions of the eosinophilic degeneration type, and in Group VI, apart from their rarity, they were completely bland and were obviously of the eosinophilic degeneration type. There may be some question as to whether or not lesions of this type should be properly considered verrucae. However, all gradations may be found between the typical extrusion lesions with pronounced inflammatory base seen in active rheumatic fever cases and those consisting solely of degenerating collagen. It does not seem advisable, therefore, to make a sharp differentiation between these lesions. In the healed cases (Group VI) these lesions

may become the seat of a secondary non-bacterial thrombotic deposit which may reach considerable size. Such secondary thrombotic verrucae have been included in the purely descriptive classification of non-bacterial thrombotic endocarditis (Gross and Friedberg [52]). Obviously, therefore, the latter designation does not exclude a rheumatic origin for the underlying condition. When definite evidence of rheumatic stigmata are present in the valves and elsewhere (auricle, rings, pericardium, and so on) the condition should be termed a rheumatic lesion, healed (Group VI) with or without superimposed non-bacterial thrombotic endocarditis.

As already shown, the proliferative and necrotic processes which are concerned with the formation of verrucae occur with predilection at the most exposed portions of the valve (closure line) as well as within culs-de-sac in which eddies or blood stasis may be present, *e.g.*, in the valve pockets and chordae tendineae insertions. It was further shown that verrucae tend to localize at the most distal portion of the valve that still presents inflammatory changes. This was best exemplified in the pulmonic cusps. As deformity of the valve tip takes place, different portions of this structure become the most conspicuous edge presented to the blood stream. As a consequence, fresh rows of verrucae are found on the newly exposed portions. Thus, in the chronic cases it was not unusual to find several distinct rows of verrucae. The oldest and generally completely organized row represented the original closure line. The other rows showed various grades of organization, the most recent and freshest verrucae being situated on what would correspond to the new closure line of the altered valve, even though no actual apposition of these parts may take place because of the stiffening and deformity. This observation immediately throws out of account any consideration which assumes that anatomical and architectural factors in the vascularization of the valve determine the site of formation of these verrucae. Indeed, as was shown particularly in Groups V and VI, such verrucous transformation may take place in valves that are completely devoid of blood vessels but which show evidence that a toxic or irritative process extended from the ring through the valve leaflet into the tip. Furthermore, the mechanism described explains the occurrence of verrucae in the valve pockets and on the chordae tendineae insertions, areas that are unquestionably free from blood vessels normally.

On comparing the incidence of verrucae in the several valves and including in these figures verrucae occurring in the pockets and on the chordae tendineae insertions, it is seen that the highest incidence was in the tricuspid valve and posterior mitral leaflet. The incidence of these lesions on the aortic valve and anterior mitral leaflet, however, was so close to this that the differences could be accounted for on a statistical basis. Clearly, however, the pulmonic valve showed the lowest incidence of verrucae. It is of interest to note that the incidence of ring and valve lesions was also decidedly lower in the pulmonic valve.

The extent of the deformity varied considerably in the several valves, being greatest in the mitral and next in the aortic, tricuspid and pulmonic, in this order. These differences occurred in spite of the fact that the acuity of the initial lesion (ring lesion) and the continued activity (incidence of verrucae) appear to be approximately the same in each of these three valves. It is interesting to speculate on the determining factors that lead to these marked differences in the subsequent development of the valvular lesion. That vascularization of the valves plays no rôle in this, is obvious for the following reasons: (1) valvulitis may occur in totally non-vascularized valves; (2) observations by Gross and Kugel,[47] which have been recently extended[50], indicate that normal valves rarely, if ever, possess blood vessels distal to the rings; (3) recent workers who have claimed a high incidence of vascularization in normal valves could show no parallel between their figures and the incidence of valvular lesions [51]; and (4) once a rheumatic process has set in, the incidence of valve vascularization is practically universal, certainly in the first five clinical groups discussed. Nevertheless, the extent of valvular damage, as is well known, differs in the several valves of the heart. On the other hand, many observations indicate that the pressure to which the valve leaflet is exposed may be an important factor in determining the extent of the valvular deformity produced. The additional trauma component of intracardiac pressure, in the presence of the rheumatic infection, can account for the considerably greater extent of the valvular deformities that occur in the left heart. The trauma of valvular apposition may explain the predilection of the valve tips as opposed to the proximal two-thirds of the leaflet for the localization of the continued fibroblastic proliferative process. Of considerable interest in this connection is the frequency

with which the tricuspid valve is the seat of verrucae in the presence of hypertension of the lesser circulation (pulmonary emphysema, mitral stenosis, and so on), even in the absence of fresh lesions on the mitral valve. The fact that the tricuspid valvular lesion is generally far more severe than the pulmonic may be accounted for by the continued infection of the tricuspid ring through the annulus extensions from the aortic root and from the mitral valve. As already shown, the pulmonic ring is not linked to these annulus extensions.

In further support of the view that intracardiac tension probably plays an important rôle in determining the progress of an initial rheumatic lesion are the figures reported by Gross and Ehrlich [40] on the incidence of Aschoff bodies in the several chambers of the heart. They showed that Aschoff bodies occurred with greatest frequency in the left ventricle, and with decreasing frequency in the right ventricle, left auricle and right auricle, in that order. This incidence of Aschoff bodies is strikingly paralleled by the tension within the respective chambers. These authors also showed that there may be differences in the incidence of Aschoff bodies within various parts of the same chamber. This suggests that there are additional factors determining local predisposition, of which we have at present no knowledge. It is not necessary to assume that the epithelium covering valve tips has especial phagocytic properties since the pressure factors discussed above can adequately explain the localization of particles (bacteria) at these sites. Finally, differences in the oxygen tension of the blood bathing the valves in the right and left hearts may also have some bearing on the progress of the lesions.

The striking differences in the inflammatory reactions within the valves as influenced by the clinical course of the disease bring up the question as to what rôle general or local tissue immunity may play in this connection. It was shown that if a patient dies during a first attack or if he suffered from only one previous attack within 1 year previous to the fatal outcome, the lesions are flagrant and differ chiefly in the nature of the blood vessels, the latter in turn being determined by the duration of the rheumatic bout. However, in the more chronic cases the inflammatory reaction is considerably subdued. It may be argued that the severity of the disease which is sufficient to lead to a fatal outcome during the first or second attack

occurring within 1 year, may be so intense that flagrant exudative phenomena are to be expected. Similarly, the fact that a patient can survive a series of rheumatic attacks or present a chronic course, may be indicative of less severe infection, *i.e.*, one that would lead to a more indolent type of reactivity in the valve. On the other hand, these differences in the severity of the reaction may indicate differences in the relative immunity of the patient to the disease. Indeed, the clinical groups into which the rheumatic cases were divided may be considered as representing various degrees of resistance to the infection. Group I represents a fulminating infection with little or no resistance. In Group II, one attack was successfully resisted for a short time only, the patient dying of either a second attack or a second exacerbation. In Group III, the first attack was even more successfully resisted; however, after a period of 2 or more years, a second attack encountered insufficient resistance and led to death. Group IV can be construed as representing partial immunity to the disease, the patient successively showing temporary phases of greater or lesser resistance to the infection. In Group V, immunity is great enough to permit of a chronic drawn-out course, without, however, complete healing. Group VI represents complete immunity to the infection with cessation of all activity. Considered in this light, the qualitative and quantitative differences in the inflammatory phenomena as observed in the various groups may well depend, at least to some extent, on such differences in immunity or reactivity. In previous reports it was shown that similar differences, corresponding to the clinical course of the disease, may be observed in the response to the rheumatic infection of the myocardial interstitium (Aschoff bodies [40]), coronary tree,[41] left auricle [43] and pericardium.[45]

The comparison of the valvular lesions occurring in the different clinical groups has already been presented in the summaries given at the end of each of these chapters. It need only be repeated that the first two groups are notable for the exudative phenomena, and the last four groups for the productive changes. Group IV occupies an intermediary position in that it represents at the same time the chronicity of the latter groups, as well as the acute exacerbations of the former. Group VI represents complete healing of the rheumatic process or cessation of the rheumatic lesions. As such, the findings in this latter group are of extreme importance in that they disclose

the stigmata of the completely involuted rheumatic processes in the valve leaflets.

In conclusion, attention should be drawn to the additional gross lesions described in this report. Until comparatively recently the only gross rheumatic lesions known to occur in the heart were those due to the acute and healing stages of pericardial inflammation, the fresh and healed verrucae on the closure line and chordae tendineae insertions of the valves, the valvular deformities with the characteristic commissural agglutinations of the aortic cusps, the thickening of the chordae tendineae, the regurgitant endocardial pockets (also occurring in other conditions), the auricular endocardial lesions and, rarely, the macroscopic Aschoff bodies. To these there have been recently added the lesions at the roots of the great vessels (Gross [42]) which produce dimpling in the sinus pockets (Fig. 10) and the thickening and prominence of the subvalvular angles and ring regions (Gross and Friedberg [46]). In the present communication, descriptions are given of the more minute topographical changes found in the valves, including elongation of the auriculoventricular leaflets, with obliteration of their normal scalloping, the ham shaped chordae tendineae insertions, the approximation of the semilunar folds of the semilunar cusps to the free edges and their disappearance, the notching, entropion and ectropion of the semilunar cusps, the characteristic pocket lesions consisting of verrucous, polypoid and nodular formations, and the agglutinations and rounding of the auriculoventricular valve pockets with obliteration of their sharp angle. A description of the pathogenesis of these lesions, their life cycles and their incidence in the various clinical subdivisions of rheumatic fever is given.

Summary and Conclusions

There have been described in this report the incidence and gross and microscopic appearance of lesions in the valves, valve pockets and chordae tendineae occurring in 97 cases of rheumatic fever. These cases have been divided into six clinical groups which represent various courses taken by this disease. It has been shown that each group presents certain gross and microscopic features that bear a relation to the clinical grouping. Anatomical evidence is presented which suggests that the course taken by the disease as well as the response of the tissue may be determined by the relative state of

immunity. This does not, however, imply that rheumatic fever is primarily an allergic disease. New macroscopic and microscopic data are presented on the development of the rheumatic lesions in the valves, and a discussion is given of the factors which determine the spread of infection, the localization of the verrucous and other lesions, the extent of the valvular damage and the pathogenesis of the characteristic deformities of the valvular apparatus. Certain stigmata of the rheumatic process occurring in completely healed valves are described. These supply additional data which are of value in elucidating the pathogenesis of other cardiac lesions. A description is also given of the changes that take place in non-rheumatic valves during the first eight decades of life.

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DESCRIPTION OF PLATES

Plate 146

Fig. 1. Cross-section of normal posterior mitral leaflet. Age 8 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

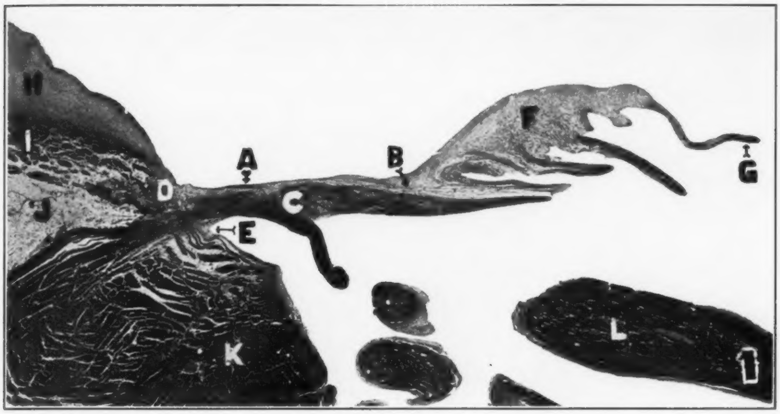
A = auricularis layer discernible only as a delicate elastic lamella; B = spongiosa layer; C = fibrosa layer; D = valve ring; E = valve pocket; F = gelatinous valve tip; G = first order chorda tendinea insertion; H = left auricular endocardium; I = left auricular myocardial wedge; J = left auricular pericardial wedge; K = left ventricular myocardium; L = columna carnea.

Fig. 2. Cross-section of normal posterior aortic cusp (to the right of the lower arrow point) and normal anterior mitral leaflet (to the left of the upper arrow point). Age 8 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = proximal layer of valve; B = spongiosa layer; C = fibrosa layer; D = valve ring; E = aortic valve pocket; F = valve tip; G = second order chorda tendinea insertion; H = left auricular endocardium; I = left auricular myocardial wedge; J = left auricular pericardial wedge; K = aortic root; L = intervalvular fibrosa.

Fig. 3. Gross photograph of tricuspid valve from a case of active rheumatic fever (Group I), showing chordae tendineae agglutinations to underlying endocardium. Age 9 years.

A = right auricle; B = anterior tricuspid leaflet; C = retracted septal tricuspid leaflet; D = chordae tendineae agglutinated to ventricular wall; E = outflow tract of right ventricle.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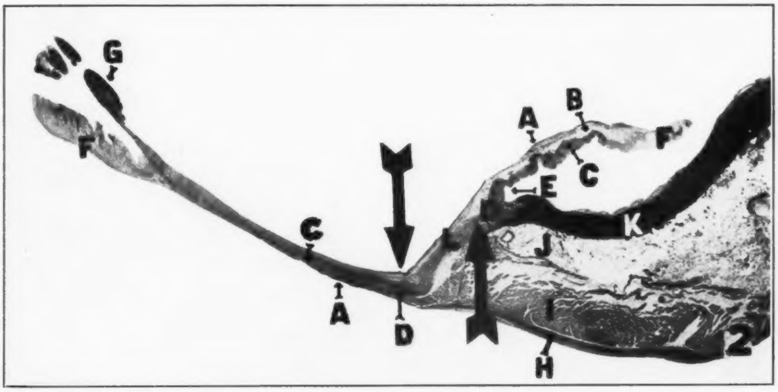
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Plate 147

Fig. 4. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group I). Age 17 months. Low power. Hematoxylin and eosin stain.

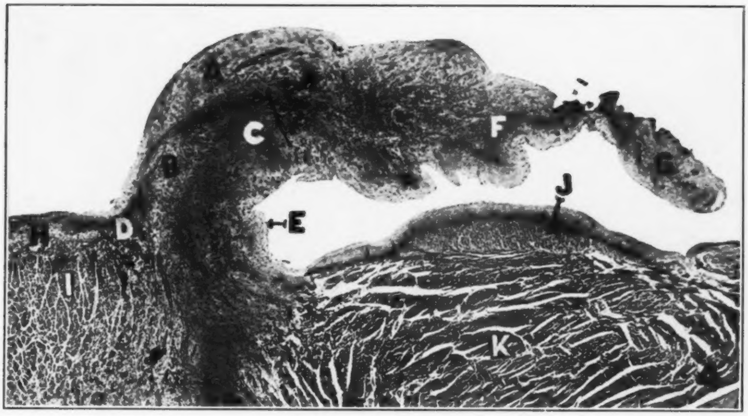
A = edematous inflamed hypercapillarized auricularis layer; B = inflamed hypercapillarized spongiosa layer; C = inflamed capillarized fibrosa layer; D = inflamed valve ring; E = inflamed capillarized pocket reduplication becoming continuous with inflamed capillarized arterialis reduplication; F = inflamed valve tip; G = inflamed, capillarized, thickened first order chorda tendinea insertion; H = inflamed left auricular endocardium (note inflamed subendocardium); I = left auricular myocardial wedge; J = left ventricular endocardial thickening; K = left ventricular myocardium.

Fig. 5. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group I). Age $2\frac{1}{2}$ years. Low power. Hematoxylin and eosin stain.

A = edematous inflamed hypercapillarized auricularis layer; B = inflamed capillarized spongiosa layer; C = inflamed capillarized fibrosa layer; D = inflamed valve ring; E = inflamed capillarized valve pocket; F = inflamed fibrotic valve tip with verrucous change; G = inflamed chordae tendineae agglutinated by verrucous material; H = inflamed left auricular endocardium (note inflamed subendocardium); I = left auricular myocardial wedge; J = inflamed thickened left ventricular endocardium; K = left ventricular myocardium.

Fig. 6. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group I). Age $6\frac{1}{2}$ years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = inflamed widened auricularis layer; B = edematous spongiosa layer; C = fibrosa layer; D = valve ring; E = inflamed reduplication in valve pocket; F = inflamed valve tip; G = cross-section of chorda tendinea showing reduplication of the endocardial covering; H = verrucous material with granulation tissue base situated on closure line (note delicate layer of fibrin attached to the surface of the verrucae); I = very delicate ventricularis reduplication; J = left ventricular myocardium.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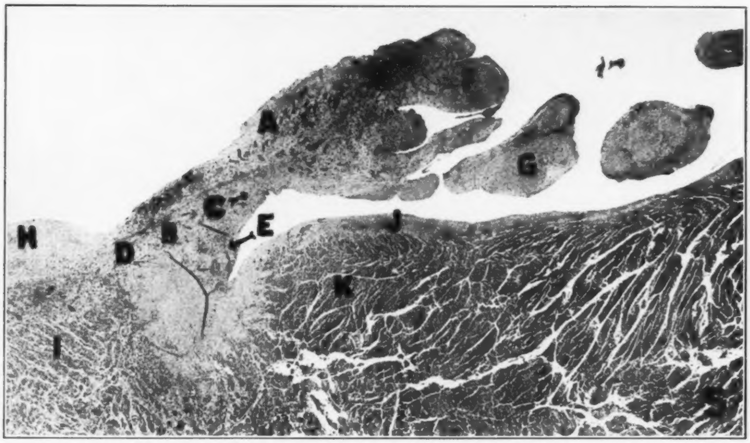
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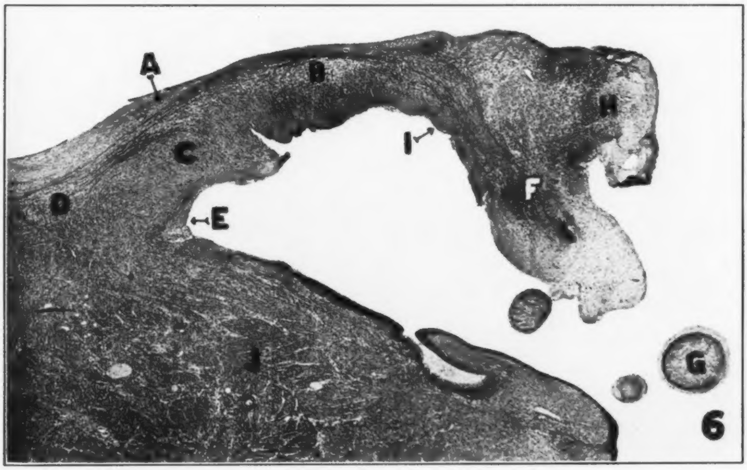
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Plate 148

Fig. 7. Cross-section of tricuspid leaflet from a case of active rheumatic fever (Group II). Age 13 years. Low power. Hematoxylin and eosin stain.

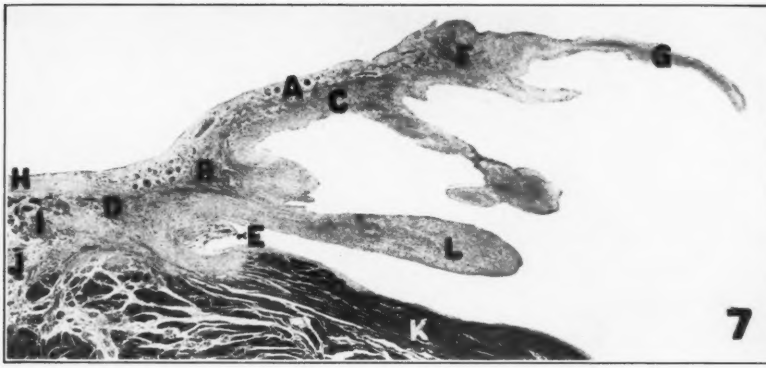
A = widened vascularized auricularis layer (note numerous intimal musculo-elastic hyperplastic vessels); B = vascularized spongiosa layer; C = fibrosa layer; D = vascularized valve ring; E = vascularized fibrotic reduplication in tricuspid pocket; F = fibrotic vascularized valve tip; G = first order chorda tendinea insertion (note fibrotic reduplication); H = left auricular endocardium; I = left auricular myocardial wedge; J = left auricular pericardial wedge; K = left ventricular myocardium; L = thickened third order chorda tendinea insertion.

Fig. 8. Cross-section of right aortic cusp from a case of active rheumatic fever (Group II). Age 18 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = elastified ventricularis reduplications; B = widened fibrotic vascularized spongiosa layer; C = fibrosa layer; D = fibrotic valve ring (note multiple elastified subaortic reduplications with intimal musculo-elastic hyperplastic vessels); E = aortic valve pocket showing polypoid formation; F = fibro-elastic transformation of valve tip with approximation of greatly thickened ventricularis layers to fibrosa and compression of spongiosa; G = fibrotic arterialis reduplication; H = verrucous lesion on closure line; I = aortic root; J = ventricular myocardium; K = considerably thickened blood vessel showing intimal musculo-elastic hyperplastic lesion.

Fig. 9. Cross-section of right aortic cusp from a case of active rheumatic fever (Group II). Age 14 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = multiple elastified ventricularis reduplications continuous with subaortic reduplications; B = fibrotic vascularized spongiosa layer; C = fibrosa layer; D = fibrotic valve ring (note multiple elastified subaortic reduplications with numerous intimal musculo-elastic hyperplastic vessels); E = aortic ring annulus permeated with intimal musculo-elastic hyperplastic vessels; F = fibro-elastic transformation of valve tip showing earliest stages of entropion; G = aortic valve pocket; H = verrucous lesion surrounding valve tip; I = aortic root; J = retroaortic pericardial mantle (note intimal musculo-elastic hyperplastic vessels); K = left auricular myocardial wedge. L = left auricular endocardium.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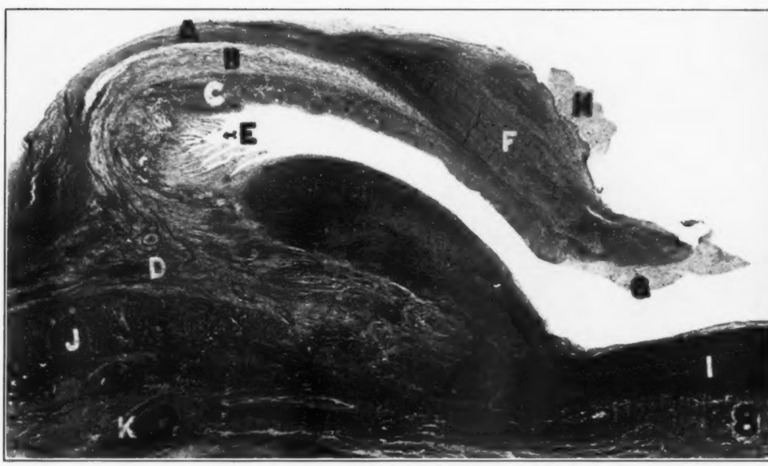
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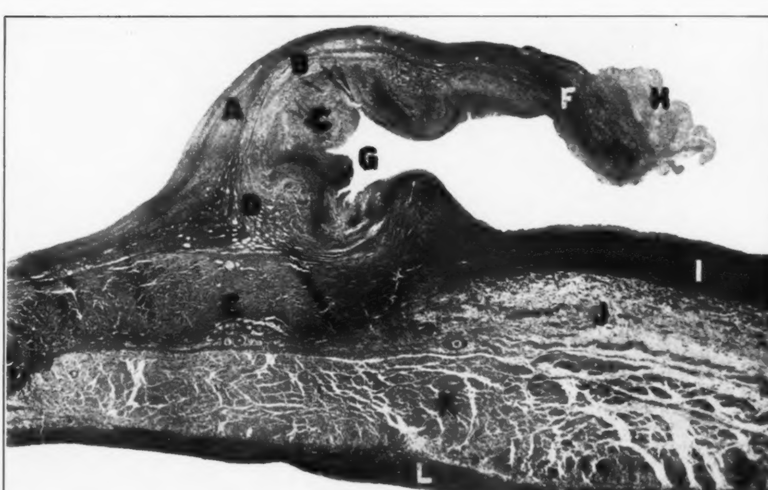
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Plate 149

FIG. 10. Gross photograph of aortic valve from a case of active rheumatic fever (Group IV). Age 12 years.

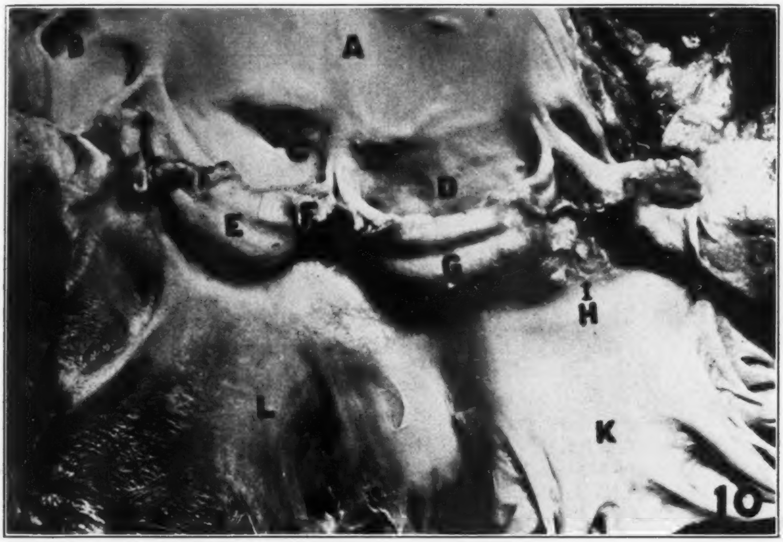
A = aorta; B = left coronary ostium; C = right coronary ostium; D = posterior sinus pocket (note dimpling of annulus); E = right aortic cusp (note notching at center of free margin and approximation of (F) semilunar folds to free margin); G = posterior aortic cusp (note rolling and thickening of free edge, beginning entropion and approximation of semilunar folds to free margin); H = irregularity of subaortic angle due to formation of subaortic angle lesions; J = bridges of verrucous material agglutinating commissure; K = ventricular aspect of anterior mitral leaflet; L = outflow tract of left ventricle.

FIG. 11. Gross photograph of mitral valve from a case of active rheumatic fever (Group IV). Age 49 years.

A = left auricle; B = anterior mitral leaflet (note gross vascularization of body of leaflet, also fresh verrucae along closure line); C = verrucae situated on chordae tendineae insertions (note ham shaped terminations of latter); D = posterior mitral leaflet with marked straightening of scalloped edge (note verrucae on closure line); E = posterior papillary muscle; F = anterior papillary muscle.

FIG. 12. Cross-section of right aortic cusp from a case of active rheumatic fever (Group IV). Age 13 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = thick fibrotic vascularized ventricularis reduplications; B = elastified compressed spongiosa layer; C = fibrosa layer; D = fibrotic valve ring (note distorted compressed capillaries); E = fibro-elastified reduplication in aortic pocket; F = ectropion of fibro-elastified valve tip; G = verrucae on new closure line; H = verrucae in cul-de-sac; I = aortic root; J = ventricular myocardium showing considerable scarring.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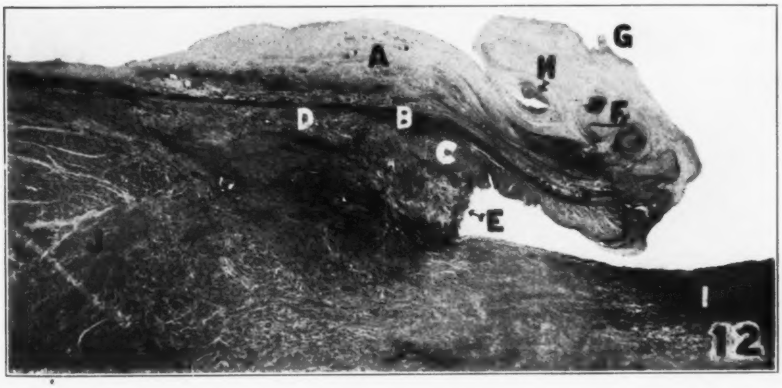
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Plate 150

Fig. 13. Cross-section of anterior mitral leaflet from a case of active rheumatic fever (Group V). Age 33 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

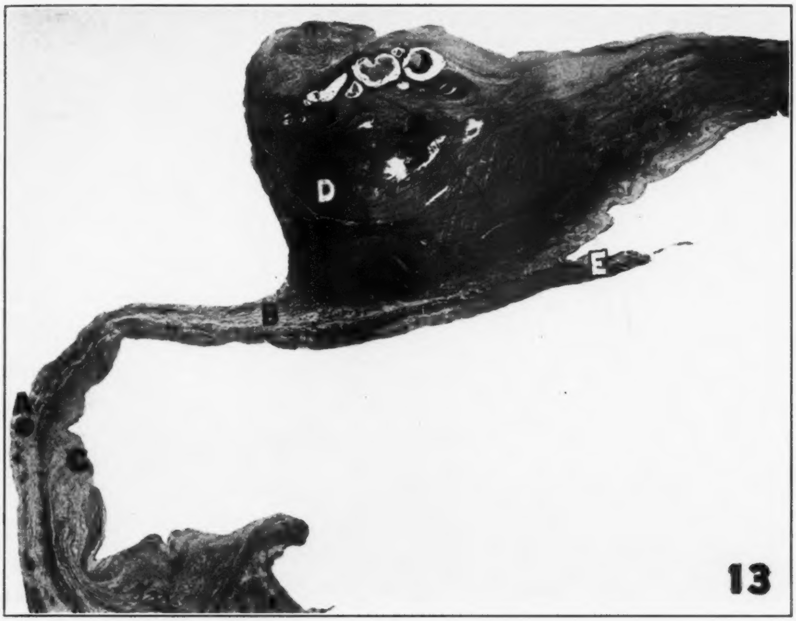
A = superficial vascularization (injected) of auricularis layer in the proximal two-thirds of the valve leaflet; B = moderately widened spongiosa layer; C = fibrosa layer; D = enormously thickened distal third of the valve leaflet. The thickening is due to fibro-elastification and fusion of auricularis and spongiosa layers together with the production of redundant elastic collagenous tissue. Note extensive vascularization of thickened tip. E = chorda tendinea insertion.

Fig. 14. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group V). Age 59 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = fused auricularis and spongiosa layers; B = chordae tendineae insertions marking the original site of the valve tip; C = enormously redundant fibrotic tissue from fused auricularis and spongiosa layers spreading over chordae tendineae and producing elongation of the valve leaflet (note absorption of chordae tendineae into this mass); D = left auricular myocardial wedge; E = left auricular pericardial wedge; F = left ventricular myocardium.

Fig. 15. Cross-section of tip of anterior mitral leaflet showing absorption of chordae tendineae and the mechanism of elongation of the leaflet. Case of active rheumatic fever (Group V). Age 66 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = tip of leaflet; B = absorbed chordae tendineae insertions; C = redundant fibro-elastic tissue from fused auricularis and spongiosa layers enveloping chordae tendineae.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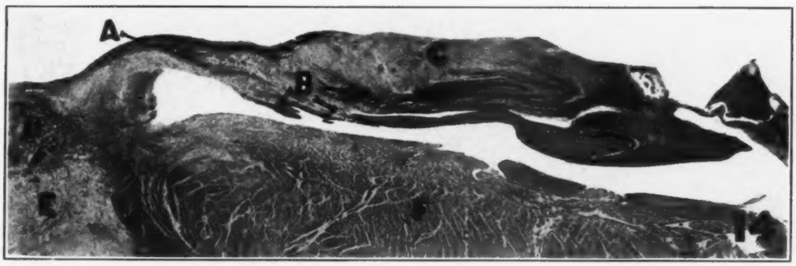
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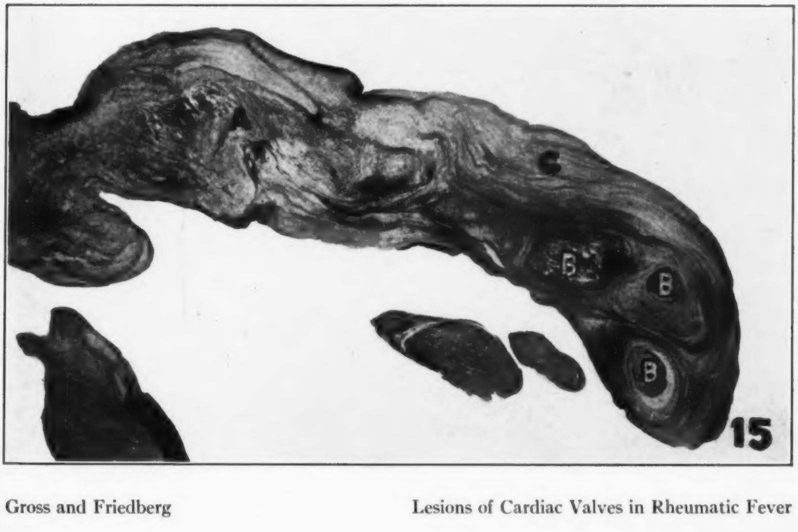
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THE OCCURRENCE OF TUMORS OF THE CENTRAL NERVOUS SYSTEM IN ROUTINE AUTOPSIES*

James H. Peers, M.D., C.M.

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Introduction

When one considers the rapidly increasing interest, both surgical and pathological, in the subject of tumors of the central nervous system during the past decade it seems truly surprising that so few surveys of a general nature have appeared. Save for Cushing's monograph,[1] and the earlier work of Bailey and Cushing[2] on which the classification at present prevailing is based, published work has dealt almost entirely with special groups of tumors, or with the clinical syndromes caused by tumors of particular localization.

From the point of view of the general pathologist something appears desirable to bridge the gap between this work of the specialist and the ordinary pathological experience of a general hospital. Cushing's great series will remain a rich mine of information for a long time to come. It is subject, however, by its manner of compilation, to certain selective influences. Based as it is on the material of a renowned neurosurgical clinic, it represents a notable preponderance of the "surgical" type of case. Also no attempt is made in it to indicate any estimate of the incidence of tumors of the nervous system in either the hospital or the general population.

Estimates of the incidence of brain tumors culled from various sources show a striking lack of agreement. Some recent surveys of the cancer problem[3,4] ignore cerebral neoplasms entirely. In contrast, perhaps the most generous estimate is that of Bailey and Cushing,[2] who state that next to the uterus the brain is the most common site of tumors. An intermediate position is taken by Ewing,[5] who believes that probably about 1 per cent of all deaths may be caused by tumors of the brain — a not inconsiderable figure. In a subject so notoriously beset by diagnostic pitfalls as is the recognition of intracranial tumors, accurate statistics must await a far more universal surgical exploration or postmortem examination than now prevails. At present we have only random samplings, chiefly in the earlier literature. Ewing[5] quotes a few of these, mostly from German publi-

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cations, indicating that brain tumors were found in from 1 to 2 per cent of all autopsies. At any rate we can agree with Bailey's more recent statement[6] that intracranial tumors are certainly a good deal more common than is usually supposed. In view of the scarcity of general surveys since the introduction of a histogenetic classification, it is felt that a study of the tumors of the central nervous system occurring in the autopsy service of a large general hospital may furnish material of some interest and value.

Material and Methods

The material for this study was drawn from the autopsies performed at the Boston City Hospital from January, 1896, through December, 1934, a total of 10,592. In 5150 of these the head was examined. Exclusive of the neoplasms of the nervous system 902 malignant tumors were found: 368 benign tumors, chiefly leiomyomas, were noted, though this figure is doubtless much too low as many small masses were probably overlooked.

From the great majority of autopsies slides of the tumors of the nervous system stained with eosin-methylene blue and with phosphotungstic acid hematoxylin were still available in the files of the Mallory Institute. The slides from a moderate number of the early autopsies had unfortunately disappeared. In a few such cases tissue was still available and this was cut and stained to permit of classification of the tumor. In the discussion of the gliomas, when neither tissue nor slides were available such tumors were listed in the unclassified group even though in a considerable number of instances the recorded description was sufficiently clear to make possible a reasonably accurate classification.

On some tumors, encountered in the last 4 years of the series, during the author's period of residence, the Spanish methods of metallic impregnation were employed with occasional helpful results. In general, however, it seemed that impregnations found their greatest utility in Bailey's pioneer work. There they furnished invaluable evidence in proving the similarity of the tumor cells in morphology and staining reaction to the already recognized stages of glial development. Now that the foregoing cell types are fairly generally recognized it is doubtful, save in selected cases, if the considerable labor required by capricious impregnation methods promises sufficient reward for the effort.

TABLE I

Total autopsies		10,592	
Heads examined		5,150	
Tumors other than those of the central nervous system:			
Malignant		902	
Benign		368	
Total		1,270	
Total tumors of the central nervous system — 188			
I. GLIOMAS		81	— 43.1 per cent
Astroblastoma	1		
Ependymoma	2		
Astrocytoma	15		
Glioblastoma multiforme	25		
Medulloblastoma	10		
Oligodendroglioma	1		
Pinealoma	1		
Spongioblastoma polare	8		
Ganglioneuroma	0		
Neuroepithelioma	0		
Unclassified gliomas	18		
II. PITUITARY ADENOMAS		6	— 3.2 per cent
Chromophobe	1		
Chromophile	4		
Mixed	1		
III. SHEATH TUMORS		22	— 11.7 per cent
Meningioma	18		
Acoustic neuroma	4		
IV. METASTATIC TUMORS		29	— 15.4 per cent
V. INVASIVE TUMORS		4	— 2.1 per cent
VI. BLOOD VESSEL TUMORS		5	— 2.6 per cent
Cystic hemangioma	2		
Racemose aneurysm	3		
VII. CONGENITAL TUMORS		8	— 4.3 per cent
Cranopharyngioma	0		
Cholesteatoma	4		
Chordoma	4		
VIII. GRANULOMATOUS TUMORS		19	— 10.1 per cent
Tuberculoma	16		
Gumma	3		
IX. SPINAL CORD TUMORS		4	— 2.1 per cent
X. UNCLASSIFIED TUMORS		10	— 5.3 per cent

As a routine method by far the best results were obtained by immediate Zenker fixation of representative blocks of fresh tissue, followed by staining with not overripe phosphotungstic acid hematoxylin. This method proved thoroughly reliable and simple, and stained sharply the nuclei, cytoplasmic outline, glia fibrils and blepharoplasts. When it was desired to bring out in greater contrast the vasculature and mesodermal elements Masson's trichrome stain on the same Zenker tissue furnished precise and colorful prepara-

tions. The prerequisite for satisfactory microscopic material is prompt and efficient fixation of small pieces of fresh tissue. The practice of hardening brains entire and sectioning them subsequently may provide somewhat neater gross museum specimens, but the price of exhibits so prepared is the irrevocable loss of all finer histological detail. If a gross specimen is desired it may be obtained by making one clean incision bisecting brain and tumor in the desired plane. One half may be preserved intact for the museum, while the remaining tissue is cut into pieces suitable for histological fixation.

The material for discussion is grouped in Table I. Save for minor differences the arrangement conforms to that in Cushing's monograph.[1] Following the precedent set by Cushing the granulomas of tuberculosis and syphilis were included with the tumors of the nervous system, though not with those of other organs. Subsequently each group of tumors will be discussed separately.

The Glioma Group

Astroblastoma

Only 1 example of this rather questionable tumor entity occurred in this series. The patient, a 49 year old female, entered the hospital complaining of cerebral symptoms of about 2 months duration, and died suddenly and unexpectedly while under observation. Autopsy revealed in the white matter of the right occipital lobe a thin walled, sharply demarcated cystic tumor, 5 cm. in diameter, filled with clear colorless fluid. The tumor pushed forward the right corpus striatum and the posterior horn of the right lateral ventricle. The wall of tumor tissue was 1 to 3 mm. thick, soft and light brown in color, with prominent small vessels coursing through it.

Microscopically the tissue was composed of a thin mat of bulky cells radiating around and attached to the numerous vessels by short stout sucker feet. The vessels had moderately thick collagenous walls and prominent, actively proliferating endothelium. Small areas of the tumor were necrotic. Mitoses were fairly numerous and glia fibrils were very scanty. Next to the brain tissue the tumor cells were smaller and their cytoplasm poorly preserved. There was no evidence of invasive growth, and the line of demarcation between tumor and brain was bordered by a narrow zone of reactionary gliosis.

This single case gives little support to the existence of the astroblastoma as a separate variety of glioma. Bailey[6] admits that the group is small and ill-defined. The cells of this tumor undoubtedly were predominantly of astroblastic form, but such cells in small groups are noted in other gliomas, and the extensive cystic degeneration in this case tends to corroborate the view of Roussy and Oberling,[7] who regard the astroblast-like tumor cells as degenerating forms of neoplastic astrocytes.

Ependymoma

Two examples of the tumor arising from ependymal cells appeared in this series, and though the number was small, the proportion of the total gliomas was almost identical with that in Cushing's[1] large collection. The 1st tumor, in a female of 20 years, arose apparently in the right lateral recess of the fourth ventricle. It extended laterally to appear beneath the meninges covering the cerebello-pontine angle and thence spread as a flattened mass anteriorly to the pons, posteriorly along the medulla, and for a short distance upward over the cerebellar surface.

In the 2nd patient, a male negro 40 years of age, a large, partly cystic tumor occupied the inferior half of the right frontal lobe, extending to the meninges, and producing some erosion of the underlying orbital roof.

Microscopically both tumors were purely of the spindle cell spongioblastic type, originally designated ependymoblastoma by Bailey and Cushing.[2] In both tumors the cells radiated characteristically about the numerous small vessels. Glia fibrils were not formed, but the cell tails stained heavily and, especially in the 2nd case, numerous blepharoplasts could be seen. In spite of their reputation for slow growth mitoses were fairly numerous in the 2nd tumor. Considerable necrosis was present but no calcification. The extension to the meninges in both instances, and the bone erosion in the 2nd, appear from the literature to have been quite unusual phenomena.

In neither case could the duration of symptoms definitely be stated. The 1st patient, after 3 days illness, was admitted unconscious and moribund with a diagnosis of tuberculous meningitis. On the 2nd patient brain tumor had been diagnosed 2 years previously at Cushing's clinic, but he refused to consent to operation and was finally admitted to the hospital comatose and in extremis.

Astrocytoma

In this series the astrocytoma formed the second largest single group of gliomas, and 15 examples were available. Proportionately they were only about two-thirds as numerous in this collection as in Cushing's[1] series, in which they constituted the largest single division of the gliomas. This difference in incidence may in part be explained by the inherent difference between surgical and postmortem material. Because of their more typical and leisurely evolution of symptoms, one might expect that astrocytomas would be relatively more often diagnosed in the operable stage and referred to a neurosurgical clinic, there to be successfully operated on and included in surgical statistics. As will again appear, this sort of natural selection will tend to increase the ratio of slowly growing and more benign tumors in neurosurgical figures and leave to the general hospital a greater proportion of rapidly growing, atypical and malignant tumors, unrecognized by the general practitioner and so not referred to a specialist.

Of the 15 astrocytomas studied, 1 was in the olivary region in a 6 year old child, 3 were cystic tumors in the cerebellum of young female patients 16 to 35 years of age, and the remaining 11 were in the cerebrum, about evenly distributed in the various lobes. All but 1 of the patients in the latter group were males, and they were considerably older, the average age being 55 years.

Microscopically the tumors of the brain stem and the cerebellum were composed entirely of well differentiated fibrous astrocytes forming abundant coarse glia fibrils. No mitoses were seen and the manner of growth was entirely expansile.

The cerebral astrocytomas were histologically a much less homogeneous group. An attempt to divide them into protoplasmic and fibrillary varieties failed, because while their cells were almost all fairly uniform, bulky and closely packed, resembling protoplasmic astrocytes, on closer inspection more or less abundant glia fibrils were invariably to be seen. Hence it seemed best to regard all of these astrocytomas of the cerebrum as tumors of mixed cell type with a preponderance of the protoplasmic form of cell. In all cases the tumors were moderately vascular, necrosis was quite extensive and hemorrhage was frequent.

In both their gross character and their microscopic appearance these cerebral astrocytomas tended to merge insensibly into the

glioblastomas, and no doubt many were on their way to assume a frankly malignant nature. However, their type cell was fairly uniform and, as with fibrosarcomas, a perhaps arbitrary division line was established, based on their apparent rate of growth as determined by the presence or absence of mitotic figures.

Viewed as a whole, the astrocytomas seemed to fall into two major divisions. In the cerebellum they occurred in younger patients as grossly cystic tumors of nearly pure fibrillary cell type. In contrast the cerebral tumors, at least in postmortem material, were found in older subjects. Histologically they were all of mixed fibrillary and protoplasmic cell type and were distinguished from the glioblastoma multiforme chiefly on the quantitative basis of slower apparent growth, as indicated by the scarcity of mitoses.

Of the 11 patients with cerebral astrocytomas 6 were diagnosed clinically, and 3 of these were operated on but removal of the tumor was found to be impossible. Three others died with a diagnosis of vascular accident, and on 2 the diagnosis was not noted. Craniotomy was performed on 1 of the 3 patients having a cerebellar tumor. The other 2, and the child with the tumor of the medulla, died soon after admission without a clinical diagnosis having been entered on the autopsy records.

Glioblastoma Multiforme

As suggested previously, autopsy figures, in contrast to surgical statistics, tend to show a relatively larger incidence of the atypical and rapidly growing malignant tumors presenting greater diagnostic difficulties during life. This hypothesis was further substantiated by the finding in this series of 25 cases of glioblastoma multiforme, constituting almost one-third of all gliomas encountered. At that the figure was still too low as perhaps some of the astrocytomas, and certainly a goodly number of the unclassified gliomas, might have been included in this division.

The cases of glioblastoma multiforme formed a quite homogeneous group. They all presented bulky subcortical tumors of the cerebral hemispheres, which often invaded the ventricles and basal ganglia. The tumors usually showed quite extensive gross degenerative changes in the form of necrosis, liquefaction and hemorrhage. Like the sarcomas of other tissues, the details of cell form varied from case to case and in different fields of the same tumor. Glia fibrils

were scanty or absent. The tumors had in common an anaplastic cell type and numerous mitotic figures, and the diagnosis seemed to rest most firmly on these latter two features, rather than on the somewhat inconstant presence of bizarre giant cells that were probably in good part a degenerative phenomenon. The tissue was richly vascular, but the thickening and proliferation of endothelium frequently described did not in this series of tumors seem to be characteristic of, or even particularly prominent in, the glioblastoma multiforme. In some instances, when necrosis had been extensive, the necrotic portion was invaded by abundant new formed connective tissue. One tumor in a younger patient clearly arose as a malignant transformation in a preexisting astrocytoma. In the great majority, however, the tumors seemed to have been malignant from the start.

All but 3 of the tumors occurred in middle aged subjects, being about evenly distributed in number in the fourth, fifth and sixth decades. Fourteen patients were males and 11 females. Brain tumor was diagnosed on 15, and craniotomy was performed on 8 of these. Five patients died with a diagnosis of cerebral hemorrhage and on 4 others the clinical diagnosis was not noted in the pathological records. One case was thought clinically to have been cerebrospinal syphilis.

Medulloblastoma

Ten examples of tumors considered to be medulloblastomas were encountered in this series. Though they constituted a slightly higher proportion of the gliomas than appeared in Cushing's[1] collection, the difference was not great enough to be significant. Seven tumors corresponded quite closely to the classical description. Five were found in children under the age of 10 years. The growth was in the cerebellum in 2 of these and in the thalamic region of the brain stem in 3. Two other cerebellar tumors occurred in patients 22 and 48 years of age.

All the tumors were moderately rapidly growing and were composed predominantly of small round or piriform cells. Formation of rosettes seldom progressed farther than the appearance of small, loose, ball-like aggregations of cells. The tumor cells aggressively invaded the adjacent brain tissue and frequently spread into the meninges.

Clinicially, 2 cases were diagnosed as probable meningitis and 4 as

tumor. Note of clinical diagnosis was lacking on 1 case. Surgical removal of the tumor was attempted without success on 3 patients.

The 3 remaining tumors were rather atypical growths found in the striate region of adults 30 to 57 years of age. Cushing[1] appears to have some doubt about the essential nature of these supposed cerebral medulloblastomas of adults. He suggests that they may be akin to the oligodendroglioma as their life history is often quite similar. Our tumors microscopically showed more extensive vascularization and degeneration than was apparent in the tumors from children, and further to corroborate Cushing's suggestion, scattered astrocytes and rare cells resembling immature oligodendroglia could be found. The bulk of the tissue, however, was scarcely distinguishable from that from the younger subjects, and it appeared that on histological evidence these few tumors from adults must be classified with the medulloblastomas.

Oligodendroglioma

Only 1 recognizable case of this rather uncommon variety of glioma appeared in this series. It occurred as a soft purplish mass largely replacing the left basal ganglia in a 13 year old girl. Microscopically the tumor consisted of closely packed, rather uniform round or oval cells, many having the classical appearance of a sharply bordered empty halo about the nucleus. Occasional mitoses and rare giant cells were seen, the latter probably surviving hypertrophied astrocytes. Penfield's second modification of the method of Hortega for oligodendroglia gave a strongly suggestive partial impregnation of the majority of the tumor cells and revealed among them many typical oligodendrocytes. No striking degenerative changes were present.

The clinical course of the patient extended over about 5 years. The symptoms consisted of headache, gradual mental change and increasingly frequent convulsions. Death occurred a short time after craniotomy and unsuccessful attempt at extirpation of the tumor.

Pinealoma

Only 1 instance, also, of this rare variety of glioma was encountered. It occurred in a 14 year old boy as a large soft mass, straddling the midline and extending into the basal ganglia on either side. Histologically the tissue was composed of masses of closely

packed, epithelial-like cells containing many mitoses. These cell masses were separated into small lobules and supported by trabeculae of delicate fibrous tissue thickly infiltrated with lymphoid cells. All sections were uniform in appearance and corresponded closely to the classical description of a rapidly growing pinealoma.

No clinical story was available but the patient bore the evidence of the difficulty of clinical localization of the tumor in the form of scars of old parietal, and old and recent cerebellar craniotomies.

Spongioblastoma Polare

Based on histological appearance, 8 tumors of this series were classified as polar spongioblastomas. This number was a considerably greater proportion — more than double in fact — than appears in Cushing's[1] collection. No explanation was apparent for this discrepancy.

Five of the tumors were found in the brain stem, from the olives to the optic chiasm, and in the cerebellum of patients from 6 to 38 years of age. Two occurred in the cerebrum of patients each 54 years of age. One, a small nodule hardly to be dignified by the name of tumor, appeared as a firm mass 1 cm. in diameter attached to the floor of the left lateral ventricle of a 45 year old man who died of delirium tremens. Six patients were males and 2 females. The presence of tumor was suspected clinically in all save the last patient, and extirpation was attempted in 4.

These spongioblastomas were quite uniform in histological structure. They were composed almost entirely of spindle cells arranged in fascicles of varying compactness and resembling in appearance those of a fibrosarcoma. Blood vessels were scanty and the tumor cells exhibited no special orientation toward them. Mitotic activity varied considerably from case to case. Invasive growth could be demonstrated in about half, and 1 tumor had spread locally into the meninges. In spite of the usual description given of this variety of glioma, several microscopically typical tumors contained considerable quantities of glia fibrils, apparently formed by the tumor cells. This active production of glia fibrils, taken in conjunction with the occurrence of a small tumor of typical structure attached to the ependyma, suggested that some, at least, of these so-called spongioblastomas may actually have been astrocytomas of the subependymal piloid type described by Roussy and Oberling.[7]

Ganglioneuroma and Neuroepithelioma

No examples of these extremely rare varieties of tumor were encountered in this series of autopsies.

Unclassified Gliomas

There remained in this series 18 tumors, mostly from the older records, which were undoubtedly gliomas but which have not been included in the preceding classification because neither tissue nor slides were available for study. However, some tentative grouping, based on the recorded descriptions, can be suggested. Eight of them were bulky, often extremely necrotic tumors in the cerebral hemispheres of adults, middle aged and older. This, taken with the recorded microscopic descriptions, would place the growths most probably in the group of the glioblastoma multiforme. One tumor, a cystic mass in the cerebellum of a young adult, was very likely a fibrous astrocytoma. The information now available on the remaining 9 cases did not permit of their classification into the subdivisions of the glioma group.

Discussion of the Glioma Group

The most interesting feature of the glioma group as a whole was that in spite of minor internal differences it formed in this postmortem series almost identically the same proportion of all tumors of the nervous system as in Cushing's large surgical collection. Within the group, with the exception of the spongioblastoma, the rarer types appeared to occur in about the same relative numbers in the two series. Of the 3 common varieties of gliomas, there was in this postmortem material a significantly larger proportion of the more atypical and malignant tumors. Finally, with the classification determined to a great extent by histological structure, there seemed to be a relatively larger number of tumors of the various cellular types found in the more unusual anatomical situations in the brain, and in patients outside of the usual age groups.

Pituitary Adenoma

Six cases of adenoma of the hypophysis were collected from the records. One tumor was a pure chromophobe adenoma, 4 were of the chromophile (eosinophile) type, and 1 was mixed, chiefly chromophobe.

The single chromophobe adenoma was found in a male 28 years old. He entered the hospital because of failing vision and signs of increasing intracranial pressure of 2 years duration, and died following an unsuccessful attempt at transfrontal removal of the tumor.

The 4 chromophile adenomas occurred in more elderly patients, 3 females and 1 male. The acromegalic habitus was described as slight to obvious in 3 subjects, and passed without notice in 1, a negro. They each died of various intercurrent diseases.

The clinical behavior of the single mixed adenoma was that of its predominant chromophobe element. The patient, a 46 year old female, sought treatment because of failing vision of 7 months duration, and died of shock following attempted transfrontal extirpation of the tumor.

The incidence of hypophyseal adenomas in this collection was rather less than half that shown in the sparse postmortem figures quoted by Ewing.[5] Their proportion of the total intracranial tumors was only one-sixth as great as in Cushing's[1] series. It is further interesting to note that the ratio of chromophile to chromophobe adenomas in the two series was almost exactly reversed. The chromophobe adenoma, growing more vigorously, tends early to produce urgent symptoms of pressure and failing vision, and hence bulks large in neurosurgical work. The leisurely and insidious chromophile adenoma, in many cases, may never rise symptomatically to the surgical level but remains part of the medical experience of a general hospital. The true general incidence of the two chief varieties of hypophyseal adenoma must therefore lie somewhere between the two extremes indicated by surgical and postmortem statistics.

Sheath Tumors

Tumors arising from the sheaths of the nervous system formed in this postmortem collection a relatively smaller group than in surgical figures.

Meningioma

Eighteen patients having meningiomas came to autopsy. Their ages varied from 37 to 84 years, with the peak of incidence in the fifth decade. Females strikingly outnumbered males, the ratio being 5 to 1. No particular site of predilection was apparent in the distribution of the tumors within the skull. It was noteworthy, how-

ever, that the growths that produced clinical symptoms were almost all located along the brain stem, and in the middle and posterior cranial fossae, where their presence produced either localizing pressure signs or obstructive hydrocephalus. Eight tumors had apparently been silent during life. Eight others probably produced symptoms, and in 3 instances the patients had been subjected to craniotomy with unsuccessful attempt at localization and removal of the tumor. Record was lacking on 2 patients and their history could not accurately be conjectured from the anatomical findings.

The tumors varied from 1 to 8 cm. in largest dimension. Microscopically they formed a fairly homogeneous group, differing only moderately in the amount of collagen they contained. A few, however, tended to be predominantly of spindle cell type, with rare giant cells and sparse mitoses.

Included in this group of meningiomas were the only 2 cases of multiple primary intracranial tumors encountered in this study. One patient, a female of 63 years, had a small meningioma embedded in the left frontal lobe and a large glioblastoma multiforme, the immediate cause of death, in the right temporal lobe. The other, a female of 83 years, had 2 small typical meningiomas, 1 in the left frontal and 1 in the right Rolandic region. Just posterior to the latter, deeply embedded in the parietal cortex, was a 3rd and larger mass. This was a rapidly growing fibroblastic tumor arising apparently from the pia-arachnoid and considered to be an atypical and rapidly growing meningioma.

Acoustic Neuroma

Only 4 instances of tumors of the acoustic nerve were found. The much greater relative number appearing in surgical studies would seem to be another instance of a tumor of slow and characteristic clinical course tending to accumulate in larger proportion at a surgical clinic.

The 4 tumors were evenly distributed between the sexes, and 2 were in the fourth and 2 in the sixth decade of life. Two were small but histologically typical growths less than 2 cm. in diameter found incidentally in patients dead of other causes. They had apparently been quite silent clinically. The other 2 tumors were clinically diagnosed and both patients died soon after suboccipital craniotomy and unsuccessful attempt at extirpation.

Metastatic Tumors

In no other group of tumors of the nervous system do surgical and postmortem statistics differ so widely as they do in the case of metastatic tumors. Neurosurgical figures give no idea of their relative frequency because, as Cushing[1] points out, patients with known metastatic tumors are rarely admitted to a neurosurgical clinic since so little can be done to help them.

Twenty-nine instances of intracranial metastases were encountered. They constituted 15.4 per cent of all tumors of the nervous system. On the other hand, 3.2 per cent of all cases of malignant disease were proved to have intracranial metastases, and this figure no doubt fell short of the real total as the head could not be examined in more than half of the total autopsies. This incidence is rather less than the average of about 5 per cent with brain involvement given by Willis[8] in his exhaustive study of the general problem of metastasis.

The frequency with which different types of primary tumors in this series gave metastases to the nervous system is exhibited in Table II.

Table II

Primary tumor	No. with brain metastases	Solitary	Multiple
Primary carcinoma of lung ..	8	2	6
Malignant melanoma	5	–	5
Carcinoma of the breast	4	1	3
Carcinoma of the colon	2	1	1
Carcinoma of the kidney	2	1	1
Chorionepithelioma	2	–	2
Carcinoma of the prostate ..	1	1	–
Carcinoma of the adrenal....	1	–	1
Hemangioendotheliosarcoma	1	–	1
Lymphatic leukemia	1	–	1
Ewing's tumor	1	1	–
Neuroblastoma.............	1	–	1
Total	29	7	22

Primary carcinoma of the lung was in this series the most frequent single source of metastases to the brain. Conversely, a large proportion of lung carcinomas gave rise to intracranial deposits. In a study of lung carcinoma based on this same series of autopsies, Olson[9] found brain metastases in 36.3 per cent of the cases in which the head was examined. Aside from its frequency, the practical necessity of considering lung carcinoma in differential diagnosis is enhanced by

the insidious course of a number of the cases. Not uncommonly the pulmonary symptoms may be trivial or absent and the patient may seek treatment for the signs and symptoms of brain tumor. Because their neurological symptoms completely overshadowed those of the primary pulmonary lesion, 3 of the 8 patients were subjected to craniotomy on a diagnosis of probable glioma.

Brief individual notes may profitably be made of certain rare cases in the metastatic group. The 2 examples of chorionepithelioma occurred in young adults, 1 male and 1 female. In the male the primary tumor was the most active component of a testicular teratoma. The testis was removed and the patient given vigorous X-ray treatment, in spite of which death occurred about a year later from cerebral and pulmonary metastases of pure chorionepitheliomatous tissue. The female patient was admitted in coma with no reliable history obtainable and autopsy was unfortunately limited to examination of the head. In both instances death appeared to have been unexpectedly sudden and due to massive hemorrhage into one of the brain metastases.

The adrenal carcinoma occurred in an elderly negro as a solitary mass in the right adrenal, with two metastases to the right parietal lobe. Quite understandably, a diagnosis of glioma was made and one mass was surgically removed. Death occurred soon after operation and the remaining mass was found at autopsy just out of range of the operative incision. The tumor was a papillary adenocarcinoma with abundant thick mucoid secretion.

The tumor designated as hemangioendotheliosarcoma was found in a female of 63 years. Clinically and pathologically the most likely primary site appeared to have been the lungs, which were found studded with bloody tumor masses 2 to 4 cm. in diameter. A few smaller masses were present in the liver and in the mucosa of the ileum. Similar masses up to 2.5 cm. in diameter were liberally scattered throughout the brain. Microscopically the tissue was richly cellular and was composed of spindle and polygonal cells interspersed with small but numerous blood channels lined with cuboidal cells. Mitotic figures were quite plentiful throughout.

No nodular deposits were present in the case of lymphatic leukemia. Instead, the entire inner surface of the dura was covered with a delicate mat of tissue from a few millimeters to a centimeter in thickness composed of closely packed small lymphocytes supported by a

very scanty vascular stroma. There was also extensive lymphoid infiltration of the pia-arachnoid, with some prolongation into the perivascular spaces but no apparent deposits in the brain substance.

In the case of the Ewing's tumor the patient, a female of 27 years, had suffered an amputation of the right leg for the primary tumor a year previously. Death was caused by a large, primarily extradural mass in the right temporal region, compressing but not invading the underlying brain. No other masses were found, either in the brain substance or in the other viscera. The tissue was microscopically typical of the endothelial myeloma of Ewing and was said in the record to be identical with the tissue from the amputated leg.

The neuroblastoma, a typical example of the sympathetic type, was encountered in a girl of 4 years. The primary tumor apparently originated in the region of the coeliac plexus and gave rise to extensive visceral metastases. The inner surface of the dura was studded with large hemispherical masses that deeply indented but did not actually invade the underlying brain.

All of the patients in this metastatic group were adults, save the child with the neuroblastoma. A significant difference in sex incidence was not apparent except in the case of the lung carcinomas, in which group males outnumbered females 3 to 1. In this series, also, the well recognized preponderance of multiple over solitary metastases was clearly demonstrated.

No case of intradural or intramedullary metastasis in the spinal cord was encountered in these autopsies.

From the foregoing discussion it is evident that intracranial metastases occur considerably more frequently in general hospital patients than would be suggested by neurosurgical statistics. It is also probable that these postmortem figures fall short of the actual incidence, since we were not permitted to examine the head in more than half of the cases of malignant disease and thereby missed a good number of metastases, both silent and suspected. Especially is attention drawn to the relatively frequent occurrence of intracranial metastases from lung carcinoma and the not uncommon tendency for the primary tumor to remain symptomatically unobtrusive. As this tumor coincides in age incidence with the commonest of the gliomas, if futile operation is to be avoided the possibility of metastasis must be considered and thoroughly ruled out in every adult tumor suspect.

Invasive Tumors

Since their manner of gaining access to the cranial cavity differs fundamentally from that involved in true metastasis, it seemed advisable to consider the invasive tumors as a separate group. As might be expected, invasive tumors were few in number, because extensive malignant tumors of the face and pharynx were relatively scarce and the skull formed a fairly effective barrier to their spread. Four such tumors were encountered. Three were carcinomas of the pharynx and antrum, extending through the base of the skull to elevate the dura and compress, but not actually invade, the overlying brain. One was a lymphoblastoma of the reticulum cell sarcoma type in a male, 26 years of age. The growth, primarily in the cervical nodes, extended into the larynx and tongue and upward through the base of the skull into the left middle cranial fossa, where it invaded superficially into the overlying temporal cortex.

The diagnosis of such invasive tumors is usually obvious on careful examination. One of the antrum growths, however, occurred in a 14 year old girl, and because of the age of the patient and the absence of local signs, save erosion of the sphenoid, craniotomy was performed on a diagnosis of possible cranopharyngioma.

Blood Vessel Tumors

Five examples of tumor arising from the cerebral blood vessels were found. Two of these were the cystic type of capillary hemangioma of the cerebellum described by Lindau. The patients were females aged 32 and 50 years, respectively. Death in both cases was due to obstructive hydrocephalus. The retinae in these subjects were not examined postmortem but the viscera showed no cysts or adenomas, such as have been occasionally described accompanying the angiomas of the nervous system.

The remaining 3 tumors were of the type called racemose aneurysm and consisted of tangled masses of grossly recognizable vessels. Two patients were males and 1 female. Their ages ranged from 22 to 50 years. The tumors were located, 1 each, in the left occipital and the right frontal lobe, and in the right hemisphere of the cerebellum. All appeared histologically to be of the arteriovenous variety with rather thick walled vessels and spotty calcification, but direct arterial connection was demonstrated only in the cerebellar tumor. No leiomyomatous nodules were seen among the vessels.

The patient with the cerebellar tumor died following thrombosis of the right middle cerebral artery. In the case of the occipital tumor death followed intraventricular rupture and hemorrhage. The patient with the right frontal tumor, a 22 year old male, had suffered 8 years from epilepsy. After a seizure that left him with a left hemiparesis he was admitted to the hospital and a right subtemporal decompression was performed. Death occurred a month later from softening and a small secondary hemorrhage into the ventricle. None of these patients exhibited angiomas elsewhere in the body.

Congenital Tumors

No case of cranopharyngioma occurred in this series of autopsies.

Four cholesteatomas were encountered. Two were in female patients and 2 in males. Their ages ranged from 19 to 53 years. All the tumors were located about the base of the brain and spread irregularly to a varying extent, insinuating themselves into available spaces and sometimes compressing but never invading the overlying brain. They consisted of a rind of rather pearly luster composed of stratified squamous epithelium and enclosing crumbly masses of keratinized epithelial cells, mingled in one instance with considerable oily brown fluid.

In only 1 patient, a male 46 years of age, did a cholesteatoma appear to have caused clinical symptoms. While undergoing treatment for arthritis he suddenly developed convulsions which became almost continuous and resulted in death 2 days later. At autopsy the tumor was found as a nodular mass 3 cm. in diameter pressed deeply into the inferior surface of the right frontal lobe.

Four chordomas were found among the patients coming to autopsy. Three of these were small, non-proliferating notochordal remnants over the basisphenoid, clinically silent, and merely incidental curiosities found in the course of routine examinations. Quite a few others were probably overlooked as they were a very inconspicuous object.

One tumor, however, in a female of 35 years was a true proliferating neoplasm and caused death. It grew as a large knobby mass projecting from the clivus, compressing and rotating the brain stem, and forcing its way into the white matter at the junction of the pons and medulla. It also eroded the posterior clinoid processes and appeared beneath the mucosa of the sphenoid sinus. Microscopically

the tissue consisted of cords and masses of typical physaliphorous cells embedded in a gelatinous matrix. In many areas the tumor cells produced coarse fibrils resembling myoglia fibrils, an appearance rarely observed. Because of its interest, a separate cytological study of this tumor is now in preparation and will be published subsequently.

Regarded as a group, the congenital tumors were usually clinically silent and appeared at autopsy as incidental findings, though some of the cholesteatomas might have caused symptoms had the patients lived longer. The reason for the absence of cases of cranopharyngioma, surgically the most frequent and important tumor of this group, was not apparent.

Granulomatous Tumors

As was true of metastatic tumors, the postmortem incidence of the so-called granulomatous tumors was much larger than their surgical occurrence. Nineteen granulomas making up slightly more than 10 per cent of all tumors were found in this series. Sixteen were tuberculomas, and 3 gummas.

The tuberculomas were about evenly distributed between the sexes, males slightly predominating. An unusually large proportion, 6 cases, were in negro patients, apparently an example of their reputed racial susceptibility to tuberculosis. The ages of the patients varied from 7 months to 60 years, but half were in the first decade of life.

In the great majority of cases the masses were about 1 cm. in diameter. Seven were solitary and 9 multiple. Of the solitary tubercles 4 were in the cerebellum, 2 in the brain stem, and 1 in the cerebrum. When multiple they involved chiefly the cerebrum, with occasionally one or more masses in the cerebellum. Tuberculous meningitis terminated 11 of the 16 cases. Six were associated with generalized miliary tuberculosis. In about half the instances the primary disease appeared in the peribronchial or abdominal lymph nodes. The others, where a source was found, were single cases of Pott's disease, enteritis, polyserositis, pulmonary tuberculosis, tuberculous pyonephrosis, and tuberculosis of the adrenals with Addison's syndrome.

Only 2 of the patients having tuberculomas were treated surgically. Cerebellar exploration was done on a child with masses in

both cerebellar hemispheres, but no attempt was made to remove them. A parietal craniotomy was performed on a man 50 years old, on a diagnosis of probable meningioma. The longitudinal fissure was found filled with large masses of tuberculous granulation tissue plastered to either side of the falx cerebri. Both patients died of tuberculous meningitis within a brief period after operation.

Three of the granulomatous tumors were gummas. Two of the patients were males, and 1 female, all in the fourth decade. Two tumors were solitary masses, one attached to the right cribriform plate, and one embedded in the right lenticular nucleus. The 3rd patient presented two masses abutting on the meninges, symmetrically placed, one on either side at the lower end of the Rolandic fissure. In all cases the masses were 2 to 3 cm. in diameter, firm, elastic and yellowish gray. The surrounding brain tissue was soft and edematous. All showed microscopically a rather ill-defined necrotic center surrounded and invaded by a mantle of vascular connective tissue thickly infiltrated with monocytes, lymphocytes and plasma cells. The cellular infiltration was strikingly perivascular in arrangement and more or less obliterative endarteritis was present.

All 3 cases of gumma were diagnosed clinically as possible gliomas, and 1 patient was subjected to a subtemporal decompression.

Spinal Cord Tumors

Four tumors of the spinal cord were found among the patients coming to autopsy. All the subjects were males. In all cases the tumor was intramedullary in position and located in the lower cervical and upper thoracic segments. In 3 patients, aged 32, 35, and 42 years, respectively, the tumors were typical slowly growing ependymomas. The 4th tumor, histologically a medulloblastoma, was found in a child of 6 who incidentally presented in addition congenital absence of the left forearm.

Unclassified Tumors

There remain for brief consideration 10 tumors that could not be placed satisfactorily in any of the preceding groups. Three of these tumors were probably small, clinically silent meningiomas, but no slides, tissue or microscopic description were available. Four others on which material and description were lacking were 1 each of a

small pineal tumor, a pituitary tumor, a probable cystic glioma obstructing the third ventricle, and a possible gumma. One tumor was originally regarded as a glioma, but autopsy was restricted to the head and the poorly preserved tissue now available resembled more some type of metastatic carcinoma.

Adequate microscopic preparations were available on the 2 remaining tumors. One, in a female patient 42 years old, was a moderately rapidly growing typical fibrosarcoma arising from the dura overlying the right temporoparietal region. The mass pressed into but did not actually invade the adjacent brain. The other tumor, found in a boy of 16, was a small cyst 13 mm. in diameter in the choroid plexus of the third ventricle. It was so situated as to act as a ball valve, occluding the aqueduct and producing a fatal hydrocephalus. Microscopically the cyst was filled with thin serous fluid. The delicate fibrous wall was infiltrated with lymphocytes and lined by one to several layers of columnar epithelium, in part ciliated.

Summary and Conclusions

1. The tumors found in the central nervous system in 10,592 autopsies performed at the Boston City Hospital over a period of 39 years have been collected and arranged according to the classification of Bailey and Cushing.

2. Regarding as clinically malignant all tumors of the central nervous system, they constituted 16.8 per cent of all malignant disease encountered in this series of autopsies.

3. The proportion of gliomas in this postmortem series was practically identical with that in Cushing's collection, but within the glioma group there was a significantly greater relative number of more rapidly growing malignant varieties.

4. Pituitary adenomas were relatively scarce in routine postmortem material, and in contrast to surgical experience the chromophile type greatly predominated.

5. The proportion of metastatic and granulomatous tumors was very high, due presumably to the absence of surgical selection of cases.

6. No significant variation in the incidence from year to year of tumors of the central nervous system could be discerned in this series.

Note: — In conclusion I wish to express my thanks to Dr. F. B. Mallory, and to Dr. Frederic Parker, Jr., for permission to use the accumulated material of the Institute, and for encouragement and advice in pursuing this study.

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ULTRACENTRIFUGATION OF INTRANUCLEAR INCLUSIONS IN THE SUBMAXILLARY GLANDS OF GUINEA PIGS AND GROUND MOLES*

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Intranuclear inclusion bodies, associated with certain virus diseases, have been considered in detail in numerous cytological investigations. Whether the inclusion bodies are derived from the virus directly or represent some product of nuclear origin is a paramount question. It is believed that information can be obtained on this subject if more is known of the physical properties of these bodies, and with this objective the relative specific gravity of different intranuclear inclusions has been determined and compared with similar physical attributes of normal nuclear elements.

The ultracentrifuge designed by Beams, Weed and Pickels,[1] and Beams and Pickels,[2] has provided a new method of approach. Lucas and Herrmann [3] used the instrument to centrifuge rabbit cornea infected with herpes virus, some results of which are shown in Figures 8 and 9. The uncentrifuged infected corneal cell (Fig. 9) shows the inclusion body of herpes in the center of the nucleus. The body is surrounded by a halo of nucleoplasm and the chromatin is marginated against the nuclear membrane. Centrifugation brings all the basophilic staining chromatin and eosin staining oxychromatin † to the centrifugal pole (Fig. 8). The nucleoplasm forms a layer on top of the chromatin, and the inclusion body, being lighter than any

* Aided by grants from the Rockefeller Foundation to Washington University for research in science and in virus diseases.

† The term oxychromatin is used here to designate the material identified in a normal nucleus by its greater affinity for acid than for basic dyes. Wilson[4] has reviewed the implications given to the term chromatin by various authors and the relation of oxychromatin to basophilic chromatin. He concludes (p. 90), ". . . it is preferable to retain the older term 'chromatin' provided we apply it to the whole stainable substance of the nucleus, whether basophilic or oxyphilic, and clearly recognize that basichromatin and oxychromatin are but passing phases, more or less marked and enduring, of one fundamental substance." The identity of the two substances is confirmed in at least one respect by the similarity of their specific gravities. Lucas and Herrmann[3] found that the two substances were intimately intermingled at the centrifugal pole; that there was no tendency for them to stratify into two layers. Luyet and Ernst,[5] who centrifuged the nuclei of plant cells, show the same result in all their figures from 2 to 23, but for some reason conclude that the basophilic chromatin is heavier.

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other substance in the nucleus, is concentrated at the centripetal pole.

It might be expected that centrifugation of submaxillary gland infected with the submaxillary virus would separate the inclusion body from chromatin and nucleoplasm in the same manner but the latter proves to respond quite differently.

Method

Glands from adult guinea pigs were whirled for an hour in the ultracentrifuge at a force of about a half million times gravity. The tissues were afterward fixed in Zenker's fluid with acetic acid. Control tissue from the submaxillary glands of the same guinea pig were divided into two portions. One portion was fixed immediately after its removal from the animal; the second portion was kept in a dish of physiological saline during the hour that centrifugation was in progress and then fixed at the same time as the centrifuged tissue. The purpose of two controls was to determine whether or not autolytic changes had altered the visible structure of the cell. It was noted by Lefevre and Curtis,[6] for example, that in the marsupium of some species of fresh-water mussels, eggs, which happened not to be fertilized, became swollen and, due to gravity, their cytosomal materials became stratified into three layers. In the present instance, however, no marked differences were visible in the control tissues fixed an hour apart and it may be concluded that the results obtained by centrifugation are not complicated by significant autolytic effects.

Observations

A normal, uninfected and uncentrifuged duct cell of the submaxillary gland of a guinea pig is shown in Figure 7. It was taken from control tissue which was fixed an hour after its removal from the body. The end of the cell adjacent to the lumen of the duct is placed toward the bottom of the plate. Figure 4 shows the effect of centrifugation on a similar cell and it is similarly oriented on the plate. The cytoplasm is little, if any, changed from its normal condition and the position of the nucleus in the cytosome remains the same with the centrifugal force employed, but the chromatin within the nucleus is concentrated at the centrifugal pole. The chromatin originally adherent to the nuclear membrane is as readily thrown down as the chromatin lying in the central part of the nucleus, which suggests

that chromatin applied to the nuclear membrane is not held there by any particular quality of adhesion. Mention will again be made of this when the behavior of chromatin in inclusion-bearing cells is discussed. The basophilic chromatin granules which are thrown to the centrifugal pole lie suspended in a menstruum of oxychromatin. Sufficient centrifugal force has not yet been attained to determine the important point whether there yet may exist a difference in the relative specific gravity of these two constituents of the normal nucleus or not. One difference between the two chromatin substances, however, seems evident, namely, that the acid staining chromatin is more adherent to the nuclear membrane than the basophilic chromatin and due to its high tenacity strands of it stretch across the nucleus parallel to the axis of centrifugal force. The same intermingling of the chromatin substances is evident in the corneal cell infected with herpes (Fig. 8), and in this case it seems evident from the work of Lucas and Herrmann [3] that oxychromatin is not a constituent of the inclusion body.

The considerable resistance offered by mammalian tissue cells stands in contrast to the more fluid condition found in certain plant cells. Luyet and Ernst [5] find in plant cells that a centrifugal force of 30,000 times gravity is sufficient to pull the nucleus into two pieces: one portion, containing the nucleoplasm, moves centripetally until it comes to lie in the upper part of the stratified cytosomal layers; the other portion, containing the chromatins, moves through the denser strata of the cytosome and rests against the cell membrane on the centrifugal side. The centrifugal force used by Luyet and Ernst on plant tissues is about one-sixteenth as great as used in the present work on mammalian cells. Beams and King obtained about the same concentration of chromatins in spinal ganglion [7] and in uterine gland cell nuclei [8] at forces of 400,000 times gravity as was obtained in the nuclei of submaxillary gland cells.

Many descriptions and illustrations have been published of submaxillary gland duct cells which contain the intranuclear inclusions characteristic of the action of the virus in this tissue (Wilson and DuBois,[9] Cole and Kuttner,[10] Kuttner,[11] Cowdry,[12] Scott,[13,14] Scott and Pruett,[15] Cowdry and Kitchen,[16] Pearson,[17] Andrewes,[18] Farber and Wolbach,[19] Thompson,[20] Kuttner and Wang,[21] Rector and Rector,[22] and Cowdry and Scott [23]). The cell and its nucleus are greatly hypertrophied. The inclusion body varies from spherical to elongate

and the chromatin is symmetrically distributed over its surface. Thompson,[20] who described intranuclear inclusions in the duct cells of the submaxillary glands of the rat, is the only investigator thus far to observe a striking asymmetrical distribution of chromatin. She states that there is a crescent of nuclear material on one side of the inclusion body, but does not mention, however, whether this asymmetry still exists when a cell is examined through the series of sections that cut it. Figure 5, an uncentrifuged duct cell containing an intranuclear inclusion, has a shape and distribution of chromatin not altogether typical but is presented to compare with Figure 6. It is typical, however, to the extent that it shows a halo around the inclusion body and that little and probably no basophilic chromatin is marginated against the nuclear membrane. Whether or not margination of chromatin is a characteristic of these cells is discussed below.

Centrifugation throws both the eosin staining inclusion body and adherent chromatin to the centrifugal pole (Figs. 3 and 6); the nuclear membrane at that pole is bulged into the cytoplasm by the mass, which indicates that the specific gravity of the mass is greater than that of the cytoplasm and is suggestive of the effects obtained by Luyet and Ernst [5] in normal plant cells. The nucleoplasm is pressed to the centripetal pole. Inclusion and chromatin must have almost identical specific gravities or else the adhesion between the two is so great that the centrifugal force employed was insufficient to separate them. Search was made for inclusions around which the chromatin was unequally distributed, as described by Thompson, in the hope that the heavier element would be indicated by its rotation to the centrifugal pole. No such inclusion was found, although from inspection of a single section the chromatin frequently appears asymmetrically distributed, but when the nucleus is traced through a series of sections the chromatin proves to be equally distributed. An example of apparent asymmetry is given in Figure 3, in which the chromatin seems to be on the centripetal side of the inclusion body, but in adjacent sections there is an equal amount laterally and on the centrifugal side. Sometimes the chromatin in the uncentrifuged cell is massed at the ends of an elongated inclusion body (Fig. 5). Centrifugation of such a cell (Fig. 6) throws the mass to one end of the nucleus and in so doing it may apparently be shortened in length, but the chromatin masses adherent to it retain their original orien-

tation. The viscous oxychromatin, seen in the uncentrifuged cell (Fig. 5) and described by Thompson as a network uniting the inclusion body and chromatin to the nuclear membrane, is stretched out by centrifugation. It still retains many points of attachment to the nuclear membrane and to the inclusion body with its adherent chromatin. Evidence that the oxychromatin is concentrated at the centrifugal pole is not as definite in the infected cell of the guinea pig as it is in the normal duct cell (Fig. 4), or in the infected cell of the ground mole (Fig. 2), perhaps, because it is not as abundant in proportion to the volume of the nucleus as it is in less hypertrophied or normal cells. Cowdry and Kitchen [16] noted radiating strands of acid staining material extending from the inclusion bodies of yellow fever to the nuclear membrane and if the inclusion is suspended by such a viscous medium it may account for the fact that Brownian movement of the granules does not occur when living cells are examined *in vitro*. On the other hand, a cytoplasmic inclusion, vaccinia, in the fragile cells of the chorio-allantoic membrane of the chick when placed in distilled water will under these conditions show rapid Brownian movement of the inclusion granules (Goodpasture, Woodruff and Buddingh [24]).

There is an important difference between the behavior of chromatin in nuclei of the cornea infected with herpes and that of nuclei of duct cells infected with submaxillary gland virus. The chromatin marginates in the former which suggests that some form of antagonism exists between it and the inclusion body. There is no margination of basophilic chromatin in the latter; all of it adheres to the inclusion body. Examination of uncentrifuged cells indicates that this is the case but centrifugation emphasizes the fact more clearly that no chromatin is present which is not attached to the inclusion body. Were some of the chromatin applied to the nuclear membrane, as it is in the normal cell nucleus (Fig. 7), or as it is in herpes-infected cells (Fig. 9), it is assumed that it would have been concentrated at the centrifugal pole in the inclusion-bearing cells of the submaxillary gland. The only marginated material in the infected duct cell displaced centrifugally independent of the inclusion body is a small quantity of acidophilic granular substance, oxychromatin.

Formed elements of the cytosome are not thrown down as readily as are nuclear structures and, with the centrifugal force employed, occur only in some of the infected cells. There seems to be no notable

shifting of cytoplasmic structures in the normal cell, nor does the nucleus as a whole change its position in relation to the cytosome. The cytosomic inclusion bodies of infected cells (Pearson [17]) are moved somewhat in a centrifugal direction (Figs. 3 and 6). The individuality of these basophilic bodies and their centrifugal displacement is shown more clearly in Giemsa stained preparations than in those used for illustration which have been stained with hematoxylin and eosin. The non-staining ground substance is somewhat concentrated at the centripetal pole.

Intranuclear inclusions in the submaxillary glands of ground moles were discovered by Rector and Rector.[22] They found the inclusions are similar in most respects to the submaxillary gland inclusions of guinea pigs; the principal difference is the basophilic character of the inclusion body in the mole.

Submaxillary glands of the mole were centrifuged in the same manner as the guinea pig tissue. The results are the same (Fig. 2): the inclusion body is thrown to the centrifugal pole and the chromatin does not separate from the inclusion body. Rector and Rector report that marginated chromatin is lacking in the inclusion-bearing cells (see also Fig. 1). The absence of marginated chromatin is confirmed when these cells are centrifuged. Some oxychromatin is brought to the centrifugal pole, which indicates that the affinities of oxychromatin and basichromatin for the inclusion body are not the same, thus pointing to a second physical difference between the two types of chromatin; the first, already mentioned, is the unequal tenacious nature of the two substances.

Discussion

The centrifugation experiments have shown that in the guinea pig chromatin of an infected submaxillary gland cell is not marginated. Cole and Kuttner [10] noted that the chromatin granules are variously distributed in the halo separating the inclusion body from the nuclear membrane. Some of these granules lie adjacent to the membrane but retain their irregular or spherical form and are not described as marginated in the same sense as is usually applied to the phenomenon of margination in cells infected with herpes or yellow fever. Farber and Wolbach [19] described the distribution of chromatin in inclusion-bearing cells of the human salivary gland in about the same terms as Cole and Kuttner, namely, that the chromatin is

concentrically arranged around the inclusion body as dense staining masses, sometimes lying in the clear zone around the inclusion and sometimes adjacent to the nuclear membrane. A similar situation exists in the infected salivary gland of the Chinese hamster, *Cricetulus griseus*, M. Edw., and in the mouse (Kuttner and Wang[21]). Thompson, studying the rat, and Rector and Rector, the mole, state that there is little or no tendency for chromatin to marginate in infected salivary gland cells of these animals. The condition described by some of the investigators who mention that some chromatin is found adjacent to the nuclear membrane may be illustrated by the isolated clump of chromatin in the lower left hand side of Figure 4. It appears to lie adjacent to the nuclear membrane, yet its adhesion to the inclusion body is evident when the cell is centrifuged. Were the chromatin not adherent to the inclusion body, it would presumably form a stratum at some level, either below or above the inclusion body. Such stratification is clearly exemplified in herpes-infected cells where margination is a definite characteristic. In the guinea pig the chromatin adheres to the surface of the inclusion body but in the mole the attraction between the two substances has gone farther and the chromatin seems to be dissolved in the inclusion body; if not actually dissolved, certainly one can safely say they are thoroughly intermingled, which indicates a compatibility between the two substances not found in viruses which produce margination. This mingling of basophilic chromatin is probably responsible for the varying degrees of basophilia described in the inclusions of submaxillary glands of various animals and man and may well be a measure of the compatibility of the two substances. That the degree of basophilia is due to different amounts of chromatin is corroborated by the faint positive reaction given by the Feulgen thymonucleic acid test for chromatin on the inclusions of guinea pigs (Cowdry[12]) and the more positive test obtained by Rector and Rector[22] for the inclusion bodies of the ground mole.

The specificity of viruses for particular animals and certain tissues is a well known distinctive characteristic of this group of pathogenic organisms. As a result of these experiments, and also from a survey of the literature, a correlation between the high specificity of submaxillary gland viruses in different animals and their close compatibility with the nuclear substance of the cells in which they occur is suggested. Demonstration of specificity

has been offered repeatedly: for example, Cole and Kuttner,[10] and Kuttner [11] were unable to transmit the submaxillary virus of the guinea pig to young rabbits, rats, kittens, chickens, pigeons, dogs or *Macacus rhesus* monkeys. Kuttner and Wang [21] were unable to transmit the submaxillary gland virus of the Chinese hamster to young guinea pigs or to rabbits. The same negative results were obtained in attempts to transmit the human submaxillary gland virus to young guinea pigs, hamsters, mice, rats, rabbits and monkeys. Likewise attempted passage from mice to young guinea pigs failed. When passage was effected from wild rats to laboratory rats only a mild reaction was obtained, demonstrating a specificity so great that animals as closely related as the two varieties of rats give different responses to the virus. Rector and Rector [22] were unable to transmit the virus from ground moles to young guinea pigs, rabbits, white mice or rats.

When the cytology of the inclusions, as found in the salivary gland duct cells of various animals, is considered it is noted that they have in common an absence of margination. It has already been noted that no one who has examined these inclusions closely describes or pictures margination of the type found in herpes and yellow fever. Centrifugation and staining techniques have demonstrated that the chromatin is associated with the inclusion bodies of submaxillary gland infections to varying degrees of intimacy.

If the suggestion is valid that the affinities and specificity of a virus are indicated by and correlated with the degree of compatibility between the inclusion body and the basophilic chromatin, then the converse should exist — namely, that viruses capable of transmission into a variety of hosts and tissues should cause margination of chromatin. Herpes is a classical example of a cosmopolitan virus producing intranuclear inclusions. It produces these inclusions in man and in many animals, such as the rabbit, guinea pig, rat, mouse, *Cebus* monkey and the chick embryo; and in many tissues, such as conjunctiva, cornea, retina, buccal mucosa, skin, trachea, liver, adrenal, ovary, testis, several different cells of the central and sympathetic nervous system,[25, 26] and fibroblasts in tissue culture.[27] The degree of pathogenicity may vary in different tissues and in different animals, but the occurrence of margination in inclusion-bearing cells always takes place. It is especially striking in those cells for which the virus does not have a natural affinity. That such is the case

is demonstrated in the colored illustration by Goodpasture and Teague [25] showing infected cells of the tracheal epithelium. The tracheal epithelium is, in a sense, a foreign host for the virus. The cytological picture expresses this relation in that margination, indicative of a chromatin-inclusion antagonism, is very pronounced. This is best shown in the younger stages where the inclusion body is small and lies in the center of a great halo created by the vigorous repulsion of the chromatin against the nuclear membrane. Goodpasture and Teague have also provided a standard of comparison, namely, a colored reproduction showing the effect of herpes on nerve cell nuclei, and in a succeeding article [28] have demonstrated the particular affinity which this virus (strain M) has for the nervous system. Since the nervous system is the "normal" habitat of this virus, just as the submaxillary gland is the "normal" habitat of the submaxillary virus, it is to be expected that the compatibility between chromatin and inclusion body would be greater than was shown in the infected epithelial cells of the trachea. That such is the case is evident when their Figure 2 is compared with Figure 1 [25]: in the nerve cell nuclei, chromatin and inclusion granules readily mingle. The inclusion is not concentrated in a compact mass in the center nor is the chromatin severely marginated.

Thus far two extremes have been considered: (1) the submaxillary gland virus group having high specificity and no margination, and (2) herpes having low specificity and pronounced margination, especially in those tissues for which it does not have a natural affinity. The specificity of many viruses lies between these two extremes and one should find intermediate stages in the degree of margination or, expressed differently, intermediate degrees of compatibility between chromatin and inclusion body. There are two viruses, yellow fever and virus III, which fall in this intermediate category about which sufficient is known cytologically to make adequate comparisons with herpes. The greater specificity of yellow fever over herpes is indicated by the few mammals aside from man which are susceptible to yellow fever, and also by the few tissues within a susceptible animal, such as the *rhesus* monkey, which will develop intranuclear inclusions. This being the case, the chromatin of a liver cell should show greater tendency toward compatibility with the inclusion body of yellow fever than of herpes. Cowdry and Kitchen [16] make these statements concerning the cytology of liver cells affected with these

viruses (page 246). "... it was found that the discrepancy in the staining properties is occasioned not by any recognizable difference in the reactions of the individual acidophilic particles which make up the inclusions, but by retention, in the early stages, of more unmarginated basophilic material in the yellow fever inclusions, as compared with those of herpes. ... The separation, or cleavage, between the acidophilic and basophilic constituents of the nucleus is therefore more marked in herpes than in yellow fever."

"... when herpetic inclusions begin to form in a localized part of the nucleoplasm they are generally limited by a halo of unstainable substance in which there is no basophilic chromatin. Halos of this kind are not so easily found in yellow fever. The usual appearance in the latter disease is represented in figures 15 and 16 where no disturbance in the distribution of the basophilic material in the immediate vicinity of the developing inclusions is at first to be detected. And ..., we may mention a distinction which is of all the easiest to make between nuclei affected by the two viruses. In the case of yellow fever the amphinucleolus generally maintains its central position in the nucleus until after the inclusions have become well developed, as is depicted in Figures 15 to 20. In herpes, on the other hand, the basophilic component is more quickly split off from the amphinucleolus, and the remaining substances become marginated on the nuclear membrane with the rest of the nuclear chromatin. For this reason central solitary nucleoli in nuclei containing mature nuclear inclusions are rare in herpes but common in yellow fever. The influence, whatever it is, which causes the margination of basophilic chromatin and the central accumulation of the acidophilic fraction sweeps through the nucleus in a more unrestrained way in herpes than in yellow fever."

Virus III likewise has a much greater animal specificity than herpes, in fact almost as great as found in submaxillary gland viruses, but does not have the same high degree of tissue specificity. In the rabbit, virus III is capable of producing intranuclear inclusions in as nearly as great a variety of tissues as herpes. Cowdry [29] notes that virus III produced inclusions in the following situations: "... (1) endothelial cells, (2) macrophages, (3) interstitial cells, (4) spermatogonia, (5) spermatocytes I and II (occasionally), and (6) epithelial cells of the tubuli recti, canals of the rete, ductuli efferentes and ductus epididymis." Rivers and Tillett,[30] Rivers and Stewart,[31] and

Miller, Andrewes and Swift [32] had previously shown that virus III will produce inclusions also in the cornea, skin, glial and nerve cells of the brain, epithelial cells of chorioid plexus, pericardium and heart muscle. Are the cytological configurations produced by virus III and herpes correspondingly proportional to the host and tissue specificity which they may possess? Cowdry [29] observed that nuclei containing herpetic inclusions are more hyperchromatic than the nuclei containing virus III inclusions. This interpreted on the basis of a "compatibility theory" means that the antagonism between inclusion and chromatin being greater in herpes, the chromatin is more vigorously concentrated against the nuclear membrane, thereby rendering its appearance more hyperchromatic. Additional confirmation is given of greater compatibility of chromatin for the virus III than for herpes inclusion body when the Feulgen thymonucleic acid test is applied.. The observed facts presented by Cowdry are: "Whether the color of the herpetic inclusions is sufficiently marked to justify the listing of the majority of herpetic inclusions as feebly positive is doubtful.

The virus III inclusions react a little more strongly. It is rare to find any which do not exhibit just a tinge of rose pink. In the case of the most compact ones, the rose pink is replaced by a light mallow purple. But this mallow purple is still very much lighter than the color taken by the nuclear chromatin. It is not unusual to find in the testicles inoculated with virus III that some of the halos separating the inclusions from the surrounding nuclear membranes are themselves evenly colored a pale rose despite the fact that they are devoid of visible contents."

There is still a wide gap between the submaxillary virus inclusions which do not produce margination and the virus of herpes, yellow fever and virus III which do. This gap can be bridged partially, at least. When the submaxillary gland virus is inoculated intracerebrally or intraperitoneally into a guinea pig some mononuclear leukocytes show intranuclear inclusions. This is not a cell for which the virus has a natural affinity and, as might be expected, lack of adaptation is expressed by an antagonism which produces margination of chromatin. It remains to be determined by centrifugation experiments to what extent this antagonism occurs in the transplantation of the virus into a foreign cell. Such experiments are already underway.

Recently Cowdry and Scott [23] have described an intranuclear in-

clusion in the submaxillary gland duct cells of the *Cebus fatuellus* monkey. From their illustrations and descriptions the reaction of the chromatin to the inclusion is an excellent example of a condition intermediate between the close compatibility of the substances found in the ground mole and guinea pig and the antagonism present in herpes. It is hoped that someone to whom a supply of living *Cebus* monkeys is available will determine the specificity of the virus producing the submaxillary gland inclusion.

It is realized that the whole field of intranuclear inclusions has not been surveyed but the discussion has been limited to those of which I have had more or less personal knowledge. It is acknowledged that before this suggested correlation between host or tissue specificity of the virus and chromatin-inclusion compatibility can attain weight as a working hypothesis, it is necessary that inclusions such as those of varicella, fox encephalitis,[33] Rift Valley fever,[34] Borna disease, poliomyelitis,[35,36] mad itch of dogs,[37] Brazilian virus,[38] and many others be considered and in some cases reëxamined cytologically in different stages of development* and subjected to centrifugation experiments. More data will have to be accumulated concerning the specificity of the virus before the validity of the suggestion will either gain credence or be unequivocally disproved.

It would seem, however, from the work done thus far that perhaps the reaction of a virus to its host tissue may ultimately find elucidation in the same fundamental biological principles that underlie the compatibility of homoplastic and heteroplastic tissue transplants and of cross-fertilization between different species and genera.

Summary and Conclusions

1. When submaxillary gland tissue is centrifuged, normal nuclei of duct epithelial cells are modified: both basi- and oxychromatin are concentrated at the centrifugal pole and the nucleoplasm, being lighter, is moved centripetally. There appears to be no difference in specific gravity between basi- and oxychromatin, but the latter resists separation from its attachments to the nuclear membrane and other objects in the nucleus.

* It is obvious that comparisons and conclusions can be made more accurately if the cytological changes are observed at various stages during the formation of the inclusion body. Where margination occurs, it is known that the development of the condition is progressive; therefore a comparison of an early stage of inclusion body formation of one virus with a late stage of another virus would obviously lead to error.

2. Duct cells of the guinea pig or ground mole containing intranuclear inclusion bodies produced by their submaxillary gland viruses respond to centrifugation by displacing both basichromatin and inclusion body to the centrifugal pole of the nucleus. It is apparent from centrifugation that the basichromatin is strongly adherent to the inclusion body. In the ground mole the association between inclusion body and chromatin is more intimate than it is in the guinea pig.

3. A correlation is suggested between the chromatin-inclusion body relation and the specificity which a virus has for a particular host or tissue. When a virus is very selective, as it is in the submaxillary viruses, generally there is found a corresponding compatibility between inclusion body and the chromatin of the infected cell. In contrast, viruses having low specificity, such as herpes, which is cosmopolitan in infective potentialities, show low compatibility with the nuclear material. This is expressed in margination of chromatin and is indicated also by the results of centrifugation experiments.

4. The literature on viruses producing intranuclear inclusions furnishes examples showing various degrees of compatibility of the chromatin for the inclusion body and of the infective specificities of those viruses for certain hosts or tissues. These examples seem to fit into the theory suggested that the cytological picture may prove to be a measure of the infective specificity of the virus and that both have a common origin in the same biological principle.

After this manuscript went to press the publication of R. G. Green[39] came to my attention. His concept of the retrogressive and adaptive evolution of viruses harmonizes closely with the work reported here. Applying his conclusion, it seems quite reasonable to interpret the different cytological pictures as expressions of the degree of adaptation that has been attained by the viruses.

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DESCRIPTION OF PLATE

PLATE 151

The magnification of the published figures is 2750 times. The direction of centrifugal force is indicated by arrows in Figures 2, 3, 4, 6 and 8. The following abbreviations are used: basich., basichromatin; cyto. incl., cytoplasmic inclusion; incl., intranuclear inclusion body; mar. basich., marginated basichromatin; nuc. m., nuclear membrane; nucpl., nucleoplasm; oxych., oxychromatin.

FIG. 1. A duct cell of the submaxillary gland of the ground mole which contains an intranuclear inclusion. The basichromatin is closely associated with the inclusion body.

FIG. 2. A cell similar to the one shown in Figure 1 and from the same gland, which has been centrifuged for an hour at about 500,000 times gravity.

FIG. 3. A duct cell of a guinea pig submaxillary gland which contains an intranuclear inclusion produced by the guinea pig submaxillary gland virus. The cell has been centrifuged for an hour. Basichromatin is not separated from the inclusion body by the treatment.

FIG. 4. An illustration of a normal duct cell of the submaxillary gland which shows the effect of centrifugation. Both basichromatin and oxychromatin are concentrated at the centrifugal pole. Part of the latter is still attached to the nuclear membrane.

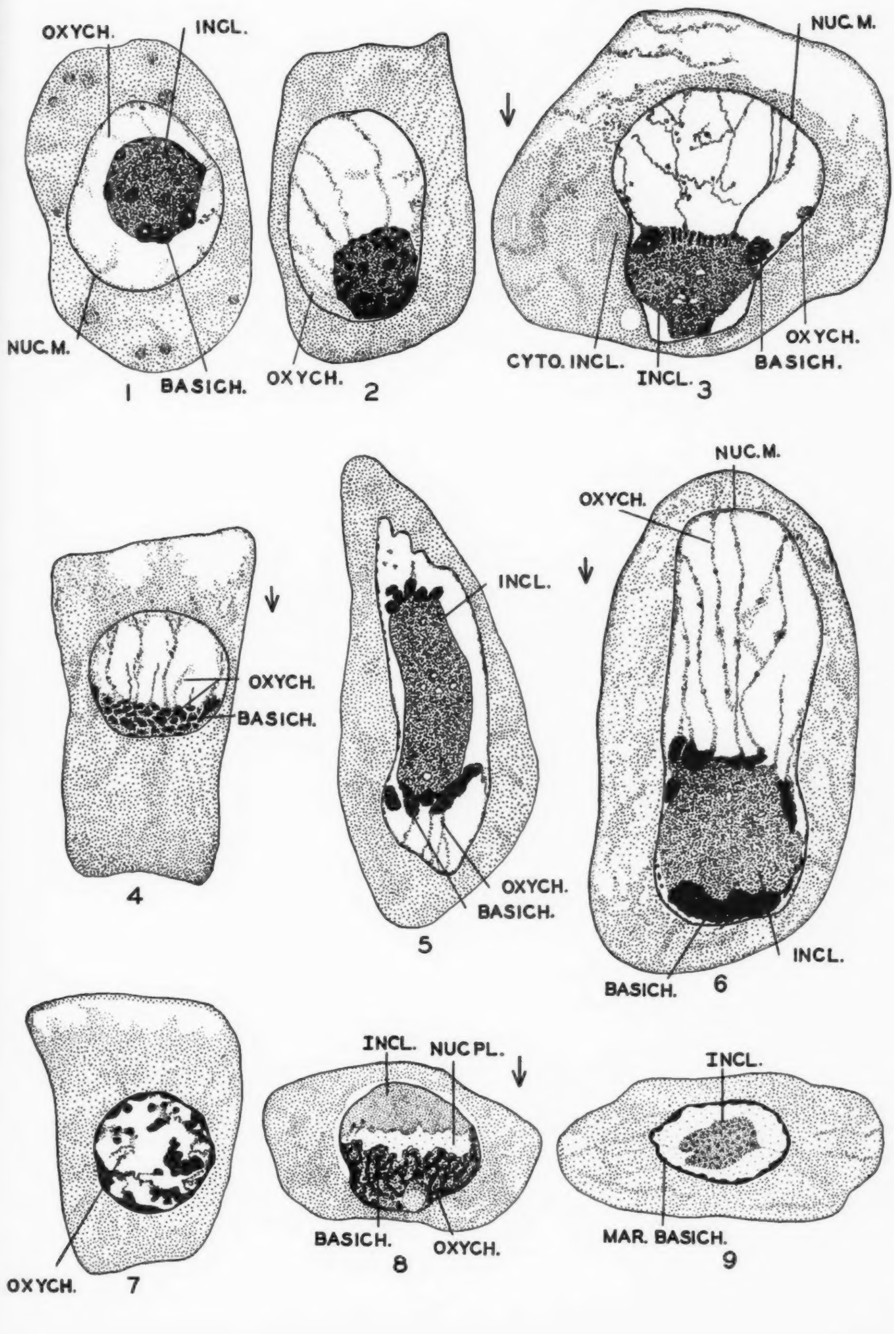
FIG. 5. An uncentrifuged infected duct cell of the submaxillary gland of the guinea pig.

FIG. 6. The effect of centrifugation on a cell similar to the one shown in Figure 5.

FIG. 7. A normal, uncentrifuged salivary duct cell shown for comparison with Figure 4 and with infected cells.

FIG. 8. A cell from the corneal epithelium of the rabbit infected with herpes virus. Centrifugation separates inclusion body, nucleoplasm and chromatin into three strata.

FIG. 9. An uncentrifuged corneal cell containing a herpes inclusion body for comparison with Figure 8.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